

Set	Items	Description
S1	16	GDP AND MANNOSE AND PYROPHOSPHORYLAS?
S2	11	RD (unique items)
S3	1	S2 AND (ANTISENS? OR RIBOZYM?)

? s b 155, 5

S4 0 B 155, 5

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PLEASE ENTER A COMMAND OR BE LOGGED OFF IN 5 MINUTES

? s gdp and mannose and pyrophosphorylas? and plant? and inhibit?

Processing

	1892	GDP
	5581	MANNOSE
	1055	PYROPHOSPHORYLAS?
	3561462	PLANT?
	279160	INHIBIT?
S5	2	GDP AND MANNOSE AND PYROPHOSPHORYLAS? AND PLANT? AND INHIBIT?

? rd

>>>Duplicate detection is not supported for File 306.

>>>Duplicate detection is not supported for File 581.

>>>Records from unsupported files will be retained in the RD set.

...completed examining records

S6 1 RD (unique items)

? t s6/3,ab/all

>>>No matching display code(s) found in file(s): 306

6/3,AB/1 (Item 1 from file: 10)

DIALOG(R)File 10:AGRICOLA

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3810234 22025758 Holding Library: AGL

Antisense inhibition of the GDP-mannose pyrophosphorylase reduces the ascorbate content in transgenic plants leading to developmental changes during senescence

Keller, R. Springer, F.; Renz, A.; Kossmann, J.

Max Planck Institut, Golm, Germany.

Oxford : Blackwell Sciences Ltd.

The Plant journal : for cell and molecular biology. July 1999. v. 19 (2) p. 131-141.

ISSN: 0960-7412

DNAL CALL NO: QK710.P68

Language: English

GDP-mannose pyrophosphorylase (GMPase, EC 2.7.7.22)

catalyses the synthesis of GDP-D-mannose and represents the first committed step in the formation of all guanosin-containing sugar nucleotides found in plants which are precursors for cell wall biosynthesis and, probably more important, the synthesis of ascorbate. A full-length cDNA encoding GMPase from *S. tuberosum* was isolated. Transgenic potato plants were generated in which the GMPase cDNA was introduced in antisense orientation to the 35S promoter. Transformants with reduced GMPase activity were selected. Transgenic plants were indistinguishable from the wild-type when held under tissue culture conditions, however, a major change was seen 10 weeks after transfer into

soil. Transgenic plants showed dark spots on leaf veins and stems with this phenotype developing from the bottom to the top of the plant. In case of the line with the strongest reduction, all aerial parts finally dried out after 3 months in soil, in contrast to the wild-type plants which did not start to senesce at this time. This coincides with a reduction of ascorbate contents in the transgenic plants, which is in agreement with the recently proposed pathway of ascorbate biosynthesis. Furthermore, leaf cell walls of the transgenic potato plants had mannose contents that were reduced to 30-50% of the wild-type levels, whereas the composition of tuber cell walls was unchanged. The glycosylation pattern of proteins was unaffected by GMPase inhibition, as studied by affino blot analysis.

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? s gdp and mannose and pyrophosphorylas?

12966 GDP

31941 MANNOSE

0 PYROPHOSPHORYLAS?

S1 0 GDP AND MANNOSE AND PYROPHOSPHORYLAS?

? s gdp and mannose and pyrophosphorylas?

12966 GDP

31941 MANNOSE

1834 PYROPHOSPHORYLAS?

S2 89 GDP AND MANNOSE AND PYROPHOSPHORYLAS?

? rd

...examined 50 records (50)

...completed examining records

S3 58 RD (unique items)

? t s3/3,ab/all

3/3,AB/1 (Item 1 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

(c) format only 2000 Dialog Corporation. All rts. reserv.

10743979 21027215

L-ascorbic acid biosynthesis.

Smirnoff N

School of Biological Sciences, University of Exeter, Exeter EX4 4PS,
United Kingdom.

Vitamins and hormones (United States) 2001, 61 p241-66, ISSN
0083-6729 Journal Code: XFE

Languages: ENGLISH

Document type: Journal Article; Review; Review, Tutorial

Biosynthesis of L-ascorbate (vitamin C) occurs by different pathways in plants and mammals. Yeast contain D-erythroascorbate, a C5 analog of ascorbate. UDP-D-glucuronic acid is the precursor in mammals. Loss of UDP forms glucuronic acid/glucuronolactone. Reduction of these at C-1 then forms L-gulonic acid/L-gulono-1,4-lactone. The lactone is oxidized by a microsomal L-gulono-1,4-lactone oxidase to ascorbate. Only the L-gulono-1,4-lactone oxidase has been purified and cloned, and very little is known about the properties of the other enzymes. Plants form ascorbate from **GDP-D-mannose** via **GDP-L-galactose**, L-galactose, and L-galactono-1,4-lactone. The final oxidation of L-galactono-1,4-lactone to ascorbate is catalyzed by a mitochondrial L-galactono-1,4-lactone dehydrogenase located on the inner membrane and using cytochrome c as electron acceptor. **GDP-mannose pyrophosphorylase** and L-galactono-1,4-lactone dehydrogenase have been cloned. Yeast synthesizes D-erythroascorbate from D-arabinose and D-arabinono-1,4-lactone in a pathway analogous to that in plants. The plant, mammalian, and yeast aldono-1,4-lactone oxidase/dehydrogenases that catalyze the last step in each pathway have significant sequence homology. L-Gulono-1,4-lactone oxidase is mutated and not expressed in animals, such as primates, that have lost ascorbate biosynthesis capacity. Assessment of the literature reveals that little is known about many of the enzymes involved in ascorbate biosynthesis or about the factors controlling flux through the pathways. There is also a possibility that minor alternative pathways exist in plants and mammals.

3/3,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2000 Dialog Corporation. All rts. reserv.

10718605 20506590

Defect in cell wall integrity of the yeast *saccharomyces cerevisiae* caused by a mutation of the **GDP-mannose pyrophosphorylase** gene VIG9.

Yoda K; Kawada T; Kaibara C; Fujie A; Abe M; Hitoshi; Hashimoto; Shimizu J; Tomishige N; Noda Y; Yamasaki M

Department of Biotechnology, The University of Tokyo, Japan.
asdfg@mail.ecc.u-tokyo.ac.jp

Bioscience, biotechnology, and biochemistry (JAPAN) Sep 2000, 64 (9)
p1937-41, ISSN 0916-8451 Journal Code: BDP

Languages: ENGLISH

Document type: Journal Article

The *Saccharomyces cerevisiae* VIG9 gene encodes **GDP-mannose pyrophosphorylase**, which synthesizes **GDP-mannose** from GTP and **mannose** -1-phosphate. Although the null mutant was lethal, the *vig9* mutants so far obtained showed no growth defect but immature protein glycosylation and drug hypersensitivity. During our search for cell-wall mutants, we found a novel temperature-sensitive mutant, JS30, which required an osmotic stabilizer for viability. JS30 excreted cell surface proteins in the medium without any indication of cell lysis. Although conventional genetic analysis using mating was impossible, by detailed characterization of JS30 including an in vitro enzyme assay and nucleotide sequencing, we found the defect of JS30 was due to a mutation in the VIG9 gene. These results indicated a critical role of **GDP-mannose** in maintenance of cell-wall integrity.

3/3,AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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10688100 20534761

Cloning, expression and characterization of the pig liver **GDP-mannose pyrophosphorylase**. Evidence that **GDP-mannose** and **GDP-Glc pyrophosphorylases** are different proteins.

Ning B; Elbein AD

Department of Biochemistry and Molecular Biology, University of Arkansas for Medical Sciences, Little Rock, Arkansas 72205, USA.

European journal of biochemistry (GERMANY) Dec 2000, 267 (23)
p6866-74, ISSN 0014-2956 Journal Code: EMZ

Contract/Grant No.: HL17783, HL, NHLBI

Languages: ENGLISH

Document type: Journal Article

GDP -Man, the mannosyl donor for most Man-containing polymers is formed by the transfer of Man-1-P to GTP to form **GDP**-Man and PPi. This reaction is catalyzed by the widespread and essential enzyme, **GDP**-Man **pyrophosphorylase** (GMPP). The pig liver GMPP consists of an alpha subunit (43 kDa) and a beta subunit (37 kDa). Purified pig GMPP catalyzes the synthesis of **GDP**-Glc (from Glc-1-P and GTP) and **GDP** -Man (from Man-1-P and GTP), but has higher activity for the formation of **GDP**-Glc than for synthesis of **GDP**-Man. In the present study, we report the cloning of the cDNA for the beta subunit of GMPP, and its expression in a bacterial system resulting in the formation of active enzyme. The full length cDNA encoding the beta subunit was isolated from a porcine cDNA library, and its predicted gene product showed high amino-acid sequence homology to GMPPs from other species. The gene was expressed in *Escherichia coli* cells, and a 37-kDa protein was over-produced in these cells. This gene product reacted strongly with antibody reactive to the native beta subunit of pig GMPP. Most interestingly, this

recombinant protein had high activity for synthesizing GDP-Man (from Man-1-P and GTP), but very low activity for the formation of GDP-Glc (from Glc-1-P and GTP). Other properties of the recombinant protein were also analyzed. This study suggests that the beta subunit is the GMPP, whereas the alpha subunit, or a combination of both subunits, may have the GDP-Glc pyrophosphorylase activity.

3/3,AB/4 (Item 4 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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10607535 20431886

Suppression of sorbitol dependence in a strain bearing a mutation in the SRB1/PSA1/VIG9 gene encoding GDP-mannose pyrophosphorylase%
% by PDE2 overexpression suggests a role for the Ras/cAMP signal-transduction pathway in the control of yeast cell-wall biogenesis.

Tomlin GC; Hamilton GE; Gardner DC; Walmsley RM; Stateva LI; Oliver SG
School of Biological Sciences, 2.205 Stopford Building, University of Manchester, Oxford Road, Manchester M13 9PT, UK.

Microbiology (ENGLAND) Sep 2000, 146 (Pt 9) p2133-46, ISSN 1350-0872 Journal Code: BXW

Languages: ENGLISH

Document type: Journal Article

Complementation studies and allele replacement in *Saccharomyces cerevisiae* revealed that PSA1/VIG9, an essential gene that encodes GDP-mannose pyrophosphorylase, is the wild-type SRB1 gene. Cloning and sequencing of the *srb1-1* allele showed that it determines a single amino acid change from glycine to aspartic acid at residue 276 (*srb1*(D276)). Genetic evidence is presented showing that at least one further mutation is required for the sorbitol dependence of *srb1*(D276). A previously reported complementing gene, which this study has now identified as PDE2, is a multi-copy suppressor of sorbitol dependence and is not, as was previously suggested, the SRB1 gene. *srb* and *pde2* mutants share a number of phenotypes, including lysis upon hypotonic shock and enhanced transformability. These data are consistent with the idea that the Ras/cAMP pathway might modulate cell-wall construction.

3/3,AB/5 (Item 5 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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10586619 20374595

The VIG9 gene products from the human pathogenic fungi *Candida albicans* and *Candida glabrata* encode GDP-mannose pyrophosphorylase

Ohta A; Chibana H; Arisawa M; Sudoh M
Department of Mycology, Nippon Roche Research Center, Kamakura, Kanagawa, Japan.

Biochimica et biophysica acta (NETHERLANDS) Jul 26 2000, 1475 (3) p265-72, ISSN 0006-3002 Journal Code: AOW

Contract/Grant No.: R01AI16567, AI, NIAID

Languages: ENGLISH

Document type: Journal Article

We have identified two genomic DNA fragments from the human pathogenic fungi, *Candida albicans* (CaVIG9) and *Candida glabrata* (CgVIG9) that encode GDP-mannose pyrophosphorylase, a key enzyme for protein glycosylation. The VIG9 homologues of CaVIG9 and CgVIG9 complement an identified protein glycosylation-defective mutation, *vig9*, of *Saccharomyces cerevisiae*. The nucleotide sequences of the ORFs, which are 83 and 90% identical to that of the ScVIG9 protein, respectively, showed a predicted gene product homologous to *S. cerevisiae* GDP-mannose pyrophosphorylase. We examined the enzyme activity of a glutathione S-transferase fusion of each VIG9 gene to synthesize GDP

mannose in the cell extracts of a heterologous *Escherichia coli* expression system. We also developed a method for detecting the enzyme activity using a non-radioactive substrate that would be applicable to high throughput screening.

3/3,AB/6 (Item 6 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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10495343 20305057

Glycosylation deficiency phenotypes resulting from depletion of GDP-mannose pyrophosphorylase in two yeast species.

Warit S; Zhang N; Short A; Walmsley RM; Oliver SG; Stateva LI

Department of Biomolecular Sciences, UMIST, PO Box 88, Manchester M60 1QD, UK.

Molecular microbiology (ENGLAND) Jun 2000, 36 (5) p1156-66, ISSN 0950-382X Journal Code: MOM

Languages: ENGLISH

Document type: JOURNAL ARTICLE

The genes encoding GDP-mannose pyrophosphorylase from *Saccharomyces cerevisiae* (SRB1/PSA1) and *Candida albicans* (CaSRB1) were expressed under the control of the tightly regulated promoters of MET3 and CaMET3 respectively. Northern analysis showed that the addition of methionine effectively blocks the transcription of pMET3-SRB1/PSA1 and pCaMET3CaSRB1 expression cassettes, which had been integrated into the genomes of appropriate mutants. Methionine-mediated repression of CaSRB1 caused loss of viability in *C. albicans*, demonstrating that, as in *S. cerevisiae*, the gene is essential for growth. Depletion of GDP-mannose pyrophosphorylase had a highly pleiotropic effect in the two yeasts. The major phenotypes observed were lysis, failure of cell separation and/or cytokinesis, impaired bud growth and bud's site selection, clumping and flocculation, as well as increased sensitivity to a wide range of antifungal drugs and cell wall inhibitors, and impaired hyphal switching ability. These phenotypes resulted from defects in glycosylation, as demonstrated by reduced affinity for Alcian blue and sensitivity to hygromycin B. Our results provide new information about the roles of protein glycosylation in yeast and, in particular, the steps that require GDP-mannose in the fungal pathogen *C. albicans*.

3/3,AB/7 (Item 7 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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10403585 20223637

Lewis X structures in the O antigen side-chain promote adhesion of *Helicobacter pylori* to the gastric epithelium.

Edwards NJ; Monteiro MA; Faller G; Walsh EJ; Moran AP; Roberts IS; High NJ

1800 Stopford Building, School of Biological Sciences, The University of Manchester, University Road, Manchester M13 9PT, UK.

Molecular microbiology (ENGLAND) Mar 2000, 35 (6) p1530-9, ISSN 0950-382X Journal Code: MOM

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Helicobacter pylori NCTC11637 expresses a lipopolysaccharide (LPS) that comprises an O antigen side-chain with structural homology to the human blood group antigen Lewis X (Le(x)). The role of this molecule in adhesion of *H. pylori* to gastric epithelial cells was investigated. Mutants expressing truncated LPS structures were generated through insertional mutagenesis of *rfbM* and *galE*; genes encode GDP mannose pyrophosphorylase and galactose epimerase respectively. Compositional and structural analysis revealed that the *galE* mutant expressed a rough LPS that lacked an O antigen side-chain. In contrast, an O antigen side-chain

was still synthesized by the rfbM mutant, but it lacked fucose and no longer reacted with anti-Le(x) monoclonal antibodies (M...). The ability of these mutants to bind to paraffin-embedded sections from the antrum region of a human stomach was assessed. Adhesion of the wild type was characterized by tropic binding to the apical surface of mucosal epithelial cells and cells lining gastric pits. In contrast, both the rfbM and galE mutants failed to demonstrate tropic binding and adhered to the tissue surface in a haphazard manner. These results indicate that LPS and, more specifically, Le(x) structures in the O antigen side-chain play an important role in targeting *H. pylori* to specific cell lineages within the gastric mucosa. The role of Le(x) in this interaction was confirmed by the tropic binding of synthetic Le(x), conjugated to latex beads, to gastric tissue. The observed pattern of adhesion was indistinguishable from that of wild-type *H. pylori*.

3/3,AB/8 (Item 8 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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10311432 20157061

Identification of ascorbic acid-deficient *Arabidopsis thaliana* mutants.
Conklin PL; Saracco SA; Norris SR; Last RL
Boyce Thompson Institute for Plant Research at Cornell University,
Ithaca, New York 14853, USA. plc3@cornell.edu
Genetics (UNITED STATES) Feb 2000, 154 (2) p847-56, ISSN 0016-6731
Journal Code: FNH
Contract/Grant No.: GM18945-01, GM, NIGMS
Languages: ENGLISH
Document type: JOURNAL ARTICLE

Vitamin C (l-ascorbic acid) is a potent antioxidant and cellular reductant present at millimolar concentrations in plants. This small molecule has roles in the reduction of prosthetic metal ions, cell wall expansion, cell division, and in the detoxification of reactive oxygen generated by photosynthesis and adverse environmental conditions. However, unlike in animals, the biosynthesis of ascorbic acid (AsA) in plants is only beginning to be unraveled. The previously described AsA-deficient *Arabidopsis* mutant *vtc1* (vitamin c-1) was recently shown to have a defect in GDP-mannose pyrophosphorylase, providing strong evidence for the recently proposed role of GDP-mannose in AsA biosynthesis. To genetically define other AsA biosynthetic loci, we have used a novel AsA assay to isolate four *vtc* mutants that define three additional VTC loci. We have also isolated a second mutant allele of *VTC1*. The four loci represented by the *vtc* mutant collection have been genetically characterized and mapped onto the *Arabidopsis* genome. The *vtc* mutants have differing ozone sensitivities. In addition, two of the mutants, *vtc2-1* and *vtc2-2*, have unusually low levels of AsA in the leaf tissue of mature plants.

3/3,AB/9 (Item 9 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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10072407 98453140

Synthesis of the A-band polysaccharide sugar D-rhamnose requires Rmd and WbpW: identification of multiple AlgA homologues, WbpW and ORF488, in *Pseudomonas aeruginosa* [published erratum appears in Mol Microbiol 1999 Jan;31(1):397-8]

Rocchetta HL; Pacan JC; Lam JS
Department of Microbiology and Canadian Bacterial Diseases Network,
University of Guelph, ON.
Molecular microbiology (ENGLAND) Sep 1998, 29 (6) p1419-34, ISSN
0950-382X Journal Code: MOM
Languages: ENGLISH

Document type: JOURNAL ARTICLE

Pseudomonas aeruginosa is capable of producing various cell-surface polysaccharides including alginate, A-band and B-band lipopolysaccharides (LPS). The D-mannuronic acid residues of alginate and the D-rhamnose (D-Rha) residues of A-band polysaccharide are both derived from the common sugar nucleotide precursor GDP-D-mannose (D-Man). Three genes, *rmd*, *gmd* and *wbpW*, which encode proteins involved in the synthesis of GDP-D-Rha, have been localized to the 5' end of the A-band gene cluster. In this study, *WbpW* was found to be homologous to phosphomannose isomerases (PMIs) and GDP-mannose pyrophosphorylases

(GMPs) involved in GDP-D-Man biosynthesis. To confirm the enzymatic activity of *WbpW*, *Escherichia coli* PMI and GMP mutants deficient in the K30 capsule were complemented with *wbpW*, and restoration of K30 capsule production was observed. This indicates that *WbpW*, like *AlgA*, is a bifunctional enzyme that possesses both PMI and GMP activities for the synthesis of GDP-D-Man. No gene encoding a phosphomannose mutase (PMM) enzyme could be identified within the A-band gene cluster. This suggests that the PMM activity of *AlgC* may be essential for synthesis of the precursor pool of GDP-D-Man, which is converted to GDP-D-Rha for A-band synthesis. *Gmd*, a previously reported A-band enzyme, and *Rmd* are predicted to perform the two-step conversion of GDP-D-Man to GDP-D-Rha. Chromosomal mutants were generated in both *rmd* and *wbpW*. The *Rmd* mutants do not produce A-band LPS, while the *WbpW* mutants synthesize very low amounts of A band after 18 h of growth. The latter observation was thought to result from the presence of the functional homologue *AlgA*, which may compensate for the *WbpW* deficiency in these mutants. Thus, *WbpW* *AlgA* double mutants were constructed. These mutants also produced low levels of A-band LPS. A search of the PAO1 genome sequence identified a second *AlgA* homologue, designated ORF488, which may be responsible for the synthesis of GDP-D-Man in the absence of *WbpW* and *AlgA*. Polymerase chain reaction (PCR) amplification and sequence analysis of this region reveals three open reading frames (ORFs), *orf477*, *orf488* and *orf303*, arranged as an operon. ORF477 is homologous to initiating enzymes that transfer glucose 1-phosphate onto undecaprenol phosphate (Und-P), while ORF303 is homologous to L-rhamnosyltransferases involved in polysaccharide assembly. Chromosomal mapping using pulsed field gel electrophoresis (PFGE) and Southern hybridization places *orf477*, *orf488* and *orf303* between 0.3 and 0.9 min on the 75 min map of PAO1, giving it a map location distinct from that of previously described polysaccharide genes. This region may represent a unique locus within *P. aeruginosa* responsible for the synthesis of another polysaccharide molecule.

3/3,AB/10 (Item 10 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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10053796 99407267

Antisense inhibition of the GDP-mannose pyrophosphorylase reduces the ascorbate content in transgenic plants leading to developmental changes during senescence.

Keller R; Renz FS; Kossmann J

Max-Planck-Institut für Molekulare Pflanzenphysiologie, Golm/Potsdam, Germany.

Plant journal (ENGLAND) Jul 1999, 19 (2) p131-41, ISSN 0960-7412
Journal Code: BRU

Languages: ENGLISH

Document type: JOURNAL ARTICLE

GDP-mannose pyrophosphorylase (GMPase, EC 2.7.7.22) catalyses the synthesis of GDP-D-mannose and represents the first committed step in the formation of all guanosin-containing sugar nucleotides found in plants which are precursors for cell wall biosynthesis and, probably more important, the synthesis of ascorbate. A full-length cDNA encoding GMPase from *S. tuberosum* was isolated. Transgenic potato plants were generated in which the GMPase cDNA was introduced in antisense

orientation to the 35S promoter. Transformants with reduced GMPase activity were selected. Transgenic plants were indistinguishable from the wild-type when held under tissue culture conditions, however, a major change was seen 10 weeks after transfer into soil. Transgenic plants showed dark spots on leaf veins and stems with this phenotype developing from the bottom to the top of the plant. In case of the line with the strongest reduction, all aerial parts finally dried out after 3 months in soil, in contrast to the wild-type plants which did not start to senesce at this time. This coincides with a reduction of ascorbate contents in the transgenic plants, which is in agreement with the recently proposed pathway of ascorbate biosynthesis. Furthermore, leaf cell walls of the transgenic potato plants had mannose contents that were reduced to 30-50% of the wild-type levels, whereas the composition of tuber cell walls was unchanged. The glycosylation pattern of proteins was unaffected by GMPase inhibition, as studied by affino blot analysis.

3/3,AB/11 (Item 11 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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09924172 99226241

Pattern of changes in the activity of enzymes of GDP-D-mannuronic acid synthesis and in the level of transcription of algA, algC and algD genes accompanying the loss and emergence of mucoidy in *Pseudomonas aeruginosa*.

Tavares IM; Leitao JH; Fialho AM; Sa-Correia I
Centro de Engenharia Biologica e Quimica, Instituto Superior Tecnico, Lisbon, Portugal.

Research in microbiology (FRANCE) Mar 1999, 150 (2) p105-16, ISSN 0923-2508 Journal Code: R6F

Languages: ENGLISH

Document type: JOURNAL ARTICLE

The low activity levels of the four GDP-D-mannuronic acid-forming enzymes, even in highly alginate-producing strains of *Pseudomonas aeruginosa*, have made it difficult to compare enzyme activities accompanying the loss/acquisition of mucoidy. Using optimized conditions, we compared the specific activity of these enzymes in three different mucoid *P. aeruginosa* cystic fibrosis isolates, in their nonmucoid spontaneous variants, and in mucoid variants that emerged during extended incubation of these nonmucoid forms in acetamide broth. A correlation was established between the promptness of emergence of the mucoid forms and the differing sensitivity to nutrient-limitation-induced death of the nonmucoid compared with the isogenic mucoid population. Consistent with the undetectable levels of algD mRNA in nonmucoid forms and with the concept that the step catalyzed by the algD-encoded GDP-mannose dehydrogenase (GMD) is a key step in control of the alginate pathway, GMD activity was undetectable or showed negligible values in nonmucoid variants and correlated with alginate production. However, phosphomannose isomerase (PMI), phosphomannomutase (PMM), and GDP-mannose pyrophosphorylase (GMP) activities in the nonmucoid forms were only slightly (40-70%) below the values in the mucoid forms. Nevertheless, no transcripts homologous to algA (encoding a bifunctional enzyme that possesses both PMI and GMP activities) were detected in the nonmucoid form, and the levels of algC (encoding PMM) transcripts, although detectable in the nonmucoid variants, were, in general, much higher in the mucoid forms. These apparently intriguing observations were cleared up by the identification of two algA functional homologues in *P. aeruginosa*, recently reported by others, and by the identification of one algC homologue, in contig225 of the PAO1 genome sequence, defining a polypeptide with a deduced amino acid sequence that showed significant homology with that of enzymes of the phosphohexomutase family found in databases. Results are also consistent with the requirement of PMI, GMP and PMM activities for the supply of GDP-D-mannose to (at least) A-band lipopolysaccharide synthesis, while GMD channels this precursor into the alginate pathway.

3/3,AB/12 (Item 12 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2000 Dialog Corporation. All rts. reserv.

09882870 99199334

Genetic evidence for the role of GDP-mannose in plant ascorbic acid (vitamin C) biosynthesis.

Conklin PL; Norris SR; Wheeler GL; Williams EH; Smirnoff N; Last RL
Boyce Thompson Institute for Plant Research and Section of Genetics and Development, Cornell University, Ithaca, NY 14853-1801, USA.
PLjC3@cornell.edu

Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Mar 30 1999, 96 (7) p4198-203, ISSN 0027-8424
Journal Code: PV3

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Vitamin C (L-ascorbic acid; AsA) acts as a potent antioxidant and cellular reductant in plants and animals. AsA has long been known to have many critical physiological roles in plants, yet its biosynthesis is only currently being defined. A pathway for AsA biosynthesis that features GDP-mannose and L-galactose has recently been proposed for plants. We have isolated a collection of AsA-deficient mutants of *Arabidopsis thaliana* that are valuable tools for testing of an AsA biosynthetic pathway. The best-characterized of these mutants (*vtc1*) contains approximately 25% of wild-type AsA and is defective in AsA biosynthesis. By using a combination of biochemical, molecular, and genetic techniques, we have demonstrated that the *VTC1* locus encodes a GDP-mannose pyrophosphorylase (mannose-1-P guanylyltransferase).

This enzyme provides GDP-mannose, which is used for cell wall carbohydrate biosynthesis and protein glycosylation as well as for AsA biosynthesis. In addition to genetically defining the first locus involved in AsA biosynthesis, this work highlights the power of using traditional mutagenesis techniques coupled with the *Arabidopsis* Genome Initiative to rapidly clone physiologically important genes.

3/3,AB/13 (Item 13 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2000 Dialog Corporation. All rts. reserv.

09813194 99145457

Purification and properties of mycobacterial GDP-mannose pyrophosphorylase.

Ning B; Elbein AD

Department of Biochemistry and Molecular Biology, University of Arkansas for Medical Sciences, Little Rock, Arkansas, 72205, USA.

Archives of biochemistry and biophysics (UNITED STATES) Feb 15 1999, 362 (2) p339-45, ISSN 0003-9861 Journal Code: 6SK

Contract/Grant No.: R03-AI43292, AI, NIAID

Languages: ENGLISH

Document type: JOURNAL ARTICLE

The enzyme that catalyzes the formation of GDP-d-mannose from GTP and alpha-d-mannose -1-P was purified about 2300-fold to near homogeneity from the soluble fraction of *Mycobacterium smegmatis*. At the final stage of purification, a major protein band of 37 kDa was observed and this band was specifically labeled, and in a concentration-dependent manner, by the photoaffinity probe 8-N3-GDP[32P]-d-mannose. The purified enzyme was stable for several months when kept in the frozen state. The 37-kDa band was subjected to protein sequencing and one peptide sequence of 25 amino acids showed over 80% identity to GDP-mannose pyrophosphorylases of pig liver and *Saccharomyces cerevisiae*. In contrast to some other bacterial GDP-mannose pyrophosphorylases, the mycobacterial enzyme was not multifunctional.

and did not have phosphomannose isomerase or phosphoglucose isomerase activity. Also, in contrast to the pig liver enzyme which uses mannose-1-P or glucose-1-P plus GTP to synthesize either GDP-mannose or GDP-glucose, the mycobacterial enzyme was specific for mannose-1-P as the sugar phosphate substrate. The enzyme was also relatively specific for GTP as the nucleoside triphosphate substrate. ITP was about 18% as effective as GTP, but ATP, CTP, and UTP were inactive. The activity of the enzyme was inhibited by GDP-glucose and glucose-1-P, although neither was a substrate for this enzyme. The pH optimum for the enzyme was 8.0, and Mg²⁺ was the best cation with optimum activity at about 5 mM. This enzyme is important for producing the activated form of mannose for formation of cell wall lipoarabinomannan and various mannose-containing glycolipids and polysaccharides. Copyright 1999 Academic Press.

3/3,AB/14 (Item 14 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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09735508 98455809

Cloning and sequencing of the *Candida albicans* homologue of SRB1/PSA1/VIG9, the essential gene encoding GDP-mannose pyrophosphorylase in *Saccharomyces cerevisiae*.

Warit S; Walmsley RM; Stateva LI

Department of Biomolecular Sciences, UMIST, Manchester, UK.

Microbiology (ENGLAND) Sep 1998, 144 (Pt 9) p2417-26, ISSN 1350-0872
Journal Code: BXW

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Two genomic fragments have been isolated from *Candida albicans* which strongly hybridize to SRB1/PSA1/VIG9, an essential gene which encodes GDP-mannose pyrophosphorylase in *Saccharomyces cerevisiae*. A common 2.5 kb XbaI-PstI fragment has been identified, which Southern analysis suggests is most likely unique in the *C. albicans* genome. The fragment contains an ORF, which is 82% identical and 90% homologous to the *Srb1p/Psalp/Vig9p* from *S. cerevisiae*, contains one additional amino acid at position 254 and is able to functionally complement the major phenotypic characteristics of *S. cerevisiae* *srb1* null and conditional mutations. The authors therefore conclude that they have cloned and sequenced from *C. albicans* the bona fide homologue of SRB1/PSA1/VIG9, named hereafter CaSRB1. Northern analysis data indicate that the gene is expressed in *C. albicans* under conditions of growth in the yeast and hyphal form and suggest that its expression might be regulated.

3/3,AB/15 (Item 15 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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09399533 98117046

Deletion of *algK* in mucoid *Pseudomonas aeruginosa* blocks alginate polymer formation and results in uronic acid secretion.

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Department of Microbiology and Immunology, University of Tennessee and Veterans Affairs Medical Center, Memphis 38163, USA.

Journal of bacteriology (UNITED STATES) Feb 1998, 180 (3) p634-41, ISSN 0021-9193 Journal Code: HH3

Contract/Grant No.: AI-19146, AI, NIAID

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Chronic pulmonary infection with *Pseudomonas aeruginosa* is a common and serious problem in patients with cystic fibrosis (CF). The *P. aeruginosa* isolates from these patients typically have a mucoid colony morphology due to overproduction of the exopolysaccharide alginate, which contributes to

the persistence of the organisms in the CF lung. Most of the alginate biosynthetic genes are clustered in the algD operon, located at 34 min on the chromosome. Alginate biosynthesis begins with the formation of an activated monomer, GDP-mannuronate, which is known to occur via the products of the algA, algC, and algD genes. Polymannuronate forms in the periplasm, but the gene products involved in mannuronate translocation across the inner membrane and its polymerization are not known. One locus of the operon which remained uncharacterized was a new gene called algK between alg44 and algE. We sequenced algK from the mucoid CF isolate FRD1 and expressed it in *Escherichia coli*, which revealed a polypeptide of the predicted size (52 kDa). The sequence of AlgK showed an apparent signal peptide characteristic of a lipoprotein. AlgK-PhoA fusion proteins were constructed and shown to be active, indicating that AlgK has a periplasmic subcellular localization. To test the phenotype of an AlgK-mutant, the algK coding sequence was replaced with a nonpolar gentamicin resistance cassette to avoid polar effects on genes downstream of algK that are essential for polymer formation. The algKdelta mutant was nonmucoid, demonstrating that AlgK was required for alginate production. Also, AlgK- mutants demonstrated a small-colony phenotype on L agar, suggesting that the loss of AlgK also caused a growth defect. The mutant phenotypes were complemented by a plasmid expressing algK in trans. When the algKdelta mutation was placed in an algJ::Tn501 background, where algA was not expressed due to polar transposon effects, the growth defect was not observed. AlgK- mutants appeared to accumulate a toxic extracellular product, and we hypothesized that this could be an unpolymerized alginate precursor. High levels of low-molecular-weight uronic acid were produced by the AlgK- mutant. When AlgK- culture supernatants were subjected to dialysis, high levels of uronic acids diffused out of the dialysis sac, and no uronic acids were detectable after extensive dialysis. In contrast, the mucoid wild-type strain produced only polymerized uronic acids (i.e., alginate), whereas the algKdelta algJ::Tn501 mutant produced no uronic acids. Thus, the alginate pathway in an AlgK- mutant was blocked after transport but at a step before polymerization, suggesting that AlgK plays an important role in the polymerization of mannuronate to alginate.

3/3,AB/16 (Item 16 from file: 155)
 DIALOG(R)File 155:MEDLINE(R)
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09350391 98068314

Oxygen-dependent upregulation of transcription of alginate genes algA, algC and algD in *Pseudomonas aeruginosa*.

Leitao JH; Sa-Correia I

Laboratorio de Engenharia Bioquimica/Centro de Engenharia Biologica e Quimica, Instituto Superior Tecnico, Lisboa.

Research in microbiology (FRANCE) Jan 1997, 148 (1) p37-43, ISSN 0923-2508 Journal Code: R6F

Languages: ENGLISH

Document type: JOURNAL ARTICLE

The mRNA levels of algA, algC and algD genes increased, coordinately, in cells of the highly mucoid *Pseudomonas aeruginosa* 8821M grown under increasing dissolved oxygen tensions (DOT) of up to 70% of air saturation. These genes encode the bifunctional protein with phosphomannose isomerase (PMI) and GDP-mannose pyrophosphorylase (GMP) activities (algA), the phosphomannomutase (PMM) (algC) and the GDP-mannose dehydrogenase (GMD) (algD). These four enzyme activities are necessary for the synthesis of GDP-mannuronic acid, which is the activated sugar precursor for alginate polymerization. For growth-limiting DOT--lower than 10% of air saturation--the increase in mRNA levels of algA, algC and algD with oxygen concentration was accompanied by a strong increase in the activity of the encoded enzymes and the consequent increase in alginate synthesis. However, and despite the upregulation of alginate gene transcription by DOT above 10% of air saturation, the activities of the encoded enzymes either maintained (GMP and GMD) or decreased (PMI and PMM)

their levels at high oxygen tensions, leading to a slight decrease in alginate synthesis. This has previously been attributed to the oxidative inactivation of alginate enzymes, particularly of PMM and PMI activities.

3/3,AB/17 (Item 17 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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09285839 97457201

X
Cloning of the aceF gene encoding the phosphomannose isomerase and GDP-mannose pyrophosphorylase activities involved in acetan biosynthesis in Acetobacter xylinum.

Griffin AM; Poelwijk ES; Morris VJ; Gasson MJ
Institute of Food Research, Norwich Laboratory, Colney, UK.
annette.griffin@bbsrc.ac.uk

☆
FEMS microbiology letters (NETHERLANDS) Sep 15 1997, 154 (2) p389-96,
ISSN 0378-1097 Journal Code: FML

Languages: ENGLISH

Document type: JOURNAL ARTICLE

The aceF gene from Acetobacter xylinum was identified and cloned from a genomic DNA library. The complete DNA sequence was determined and computer analysis of the translated gene sequence revealed homology with the deduced amino acid sequence of xanB from Xanthomonas campestris. Therefore aceF is likely to encode a bifunctional enzyme with mannose-6-phosphate isomerase (PMI) and GDP-mannose pyrophosphorylase (GMP) activities. PMI and GMP activities were detected in strains of Escherichia coli expressing the cloned aceF gene.

3/3,AB/18 (Item 18 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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09166931 97341166

X
☆
Saccharomyces cerevisiae VIG9 encodes GDP-mannose pyrophosphorylase, which is essential for protein glycosylation.

Hashimoto H; Sakakibara A; Yamasaki M; Yoda K
Department of Biotechnology, the University of Tokyo, Yayoi, Bunkyo-ku, Tokyo 113, Japan.

Journal of biological chemistry (UNITED STATES) Jun 27 1997, 272 (26)
p16308-14, ISSN 0021-9258 Journal Code: HIV

Languages: ENGLISH

Document type: JOURNAL ARTICLE

A genomic DNA fragment that complements a newly identified protein glycosylation-defective mutation, vig9, of Saccharomyces cerevisiae was cloned. Chromosomal integration of this fragment by homologous recombination indicated that it contains the wild type VIG9 gene. The nucleotide sequence was determined. A predicted gene product showed significant amino acid sequence homology with several bacterial enzymes that catalyze the synthesis of (deoxy)ribonucleotide diphosphate sugars from sugar phosphates and (deoxy)ribonucleotide triphosphate. We examined the enzyme activity to synthesize GDP-mannose in the cell extracts of the wild type, vig9-1 mutant, and VIG9 transformant yeasts. Reduction of the activity in the mutant cell and its restoration by VIG9 suggested that the VIG9 gene is the structural gene for GDP-mannose pyrophosphorylase of S. cerevisiae which catalyzes the production of GDP-mannose. We demonstrated the enzyme activity of Vig9 protein using a recombinant fusion protein produced in Escherichia coli.

3/3,AB/19 (Item 19 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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09011577 97020015

Genetic analysis of the transcriptional arrangement of *Azotobacter vinelandii* alginate biosynthetic genes: identification of two independent promoters.

Lloret L; Barreto R; Leon R; Moreno S; Martinez-Salazar J; Espin G; Soberon-Chavez G

Departamento de Microbiologia Molecular, Universidad Nacional Autonoma de Mexico, Morelos, Mexico.

Molecular microbiology (ENGLAND) Aug 1996, 21 (3) p449-57, ISSN 0950-382X Journal Code: MOM

Languages: ENGLISH

Document type: JOURNAL ARTICLE

The study of alginate biosynthesis, the exopolysaccharide produced by *Azotobacter vinelandii* and *Pseudomonas aeruginosa*, might lead to different biotechnological applications. Here we report the cloning of *A. vinelandii* *algA*, the gene coding for the bifunctional enzyme phosphomannose isomerase-guanosine diphospho-o-mannose pyrophosphorylase (PMI-GMP). This gene was selected by the complementation for xanthan gum production of *Xanthomonas campestris* pv. *campestris* *xanB*-mutants, which lack this enzymatic activity. The complementing cosmid clones selected, besides containing *algA*, presented a gene coding for an alginate lyase activity (*algL*), and some of them also contained *algD* which codes for GDP-mannose dehydrogenase. We present here the characterization of the *A. vinelandii* chromosomal region comprising *algD* and its promoter region, *algA* and *algL*, showing that, as previously reported for *P. aeruginosa*, *A. vinelandii* has a cluster of the biosynthetic alginate genes. We provide evidence for the presence of an *algD*-independent promoter in this region which transcribes at least *algL* and *algA*, and which is regulated in a manner that differs from that of the *algD* promoter.

3/3,AB/20 (Item 20 from file: 155)
DIALOG(R) File 155: MEDLINE(R)

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08927090 97081711

Expression, purification and characterization of recombinant phosphomannomutase and GDP-alpha-D-mannose pyrophosphorylase from *Salmonella enterica*, group B, for the synthesis of GDP-alpha-D-mannose from D-mannose.

Elling L; Ritter JE; Verseck S

Institut fur Enzymtechnologie, Heinrich-Heine-Universitat DAsseldorf im Forschungszentrum Julich, Germany.

Glycobiology (ENGLAND) Sep 1996, 6 (6) p591-7, ISSN 0959-6658
Journal Code: BEL

Languages: ENGLISH

Document type: JOURNAL ARTICLE

The genes *rfbK* and *rfbM* from the *rfb* cluster (O-antigen biosynthesis) of *Salmonella enterica*, group B, encoding for the enzymes phosphomannomutase (EC 5.4.2.8) and GDP-alpha-D-mannose pyrophosphorylase (EC 2.7.7.13) were overexpressed in *E. coli* BL21 (DE3) with specific activities of 0.1 U/mg and 0.3-0.6 U/mg, respectively. Both enzymes were partially purified to give specific activities of 0.26 U/mg and 2.75 U/mg, respectively. Kinetic characterization of the homodimeric (108 kDa) GDP-alpha-D-mannose pyrophosphorylase revealed a K_m for GTP and mannose -1-P of 0.2 mM and 0.01 mM with substrate surplus inhibition constants (K_i) of 10.9 mM and 0.7 mM, respectively. The product GDP-alpha-D-mannose gave a competitive inhibition with respect to GTP (K_i 14.7 microM) and an uncompetitive inhibition with respect to mannose -1-P (K_i 115 microM). Both recombinant enzymes were used for repetitive batch synthesis of GDP-alpha-D-mannose starting from D-mannose and GTP. In three subsequent batches 581 mg (960 mumol) GDP-alpha-D-mannose was synthesized with 80% average yield. The overall yield after product isolation was 22.9% (329 mumol, 199 mg).

3/3,AB/21 (Item 21 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2000 Dialog Corporation. All rts. reserv.

08590891 96105198

A putative pathway for perosamine biosynthesis is the first function encoded within the rfb region of *Vibrio cholerae* O1.

Stroeher UH; Karageorgos LE; Brown MH; Morona R; Manning PA
Department of Microbiology and Immunology, University of Adelaide, Australia.

Gene (NETHERLANDS) Dec 1 1995, 166 (1) p33-42, ISSN 0378-1119
Journal Code: FOP

Languages: ENGLISH

Document type: JOURNAL ARTICLE

The first four genes (rfbA,B,D,E) of the rfb region of *Vibrio cholerae* O1 are predicted to encode the enzymes required for the biosynthesis of perosamine, which constitutes the backbone structure of the O-antigen of the lipopolysaccharide. Based on homology to known proteins/protein families, the following functions are predicted: RfbA, phosphomannose isomerase-guanosine diphosphomannose pyrophosphorylase; RfbB, phosphomanno-mutase; RfbD, oxido reductase and RfbE, perosamine synthetase (amino-transferase). Thus, perosamine is synthesized from fructose 6-phosphate via the intermediates mannose 6-phosphate by RfbA, to mannose 1-phosphate by RfbB, to GDP-mannose by RfbA, to GDP-4-keto-6-dideoxymannose by RfbD and to GDP-perosamine by RfbE. This final product would then serve as the substrate for the addition of the tetronate, which could then be polymerized into the O-antigen for transfer to the lipid A plus core oligosaccharide and export to the cell surface. The organization of these genes are such that one would expect them to be translationally coupled as part of the rfb operon. However, the absence of readily detectable promoter sequences suggests low levels of transcription, in line with other studies. The nucleotide sequence of these genes is absolutely conserved in the two isolates 569B (classical, Inaba) and O17 (El Tor, Ogawa) which were expected to show maximal sequence variation. This suggests very tight constraints on the micro-evolution within these sequences.

3/3,AB/22 (Item 22 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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08311478 95297909

Growth-phase-dependent alginate synthesis, activity of biosynthetic enzymes and transcription of alginate genes in *Pseudomonas aeruginosa*.

Leitao JH; Sa-Correia I
Laboratorio de Engenharia Bioquimica, Instituto Superior Tecnico, Lisboa, Portugal.

Archives of microbiology (GERMANY) Mar 1995, 163 (3) p217-22, ISSN 0302-8933 Journal Code: 7YN

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Alginate synthesis by the highly mucoid *Pseudomonas aeruginosa* 8821 M is growth-phase-dependent, and the alginate produced per unit of biomass reaches maximum values in the deceleration phase of growth. However, the degree of polymerization increases as batch growth proceeds, reaching maximum values at the stationary phase of growth. The activity of the four enzymes leading to GDP-mannuronic acid formation, phosphomannose isomerase, phosphomannomutase, GDP-mannose pyrophosphorylase and GDP-mannose dehydrogenase peaked earlier at the late exponential phase. Growth-phase-dependent activity of alginate biosynthetic enzymes correlates with the level of transcription of the encoding alginate genes *algA*, *algC* and *algD* during growth, as indicated by Northern blot

hybridization experiments. The pattern of coordinate transcriptional growth-phase regulation of these alginate structural genes concurs with the growth-dependent transcription of the regulatory gene *algR1*.

3/3,AB/23 (Item 23 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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08225699 94214678

Genetic analysis of *Escherichia coli* 09 rfb: identification and DNA sequence of phosphomannomutase and GDP-mannose pyrophosphorylase genes.

Sugiyama T; Kido N; Komatsu T; Ohta M; Jann K; Jann B; Saeki A; Kato N
Department of Bacteriology, Nagoya University School of Medicine, Aichi, Japan.

Microbiology (ENGLAND) Jan 1994, 140 (Pt 1) p59-71, ISSN 1350-0872
Journal Code: BXW

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Subcloning, transposon insertion, and deletion analysis revealed that the *Escherichia coli* 09 rfb region is about 12 kb in size. The region encodes at least seven polypeptides of 89, 74, 55, 50, 44, 41 and 39.5 kDa. Southern hybridization analysis of rfb regions of *E. coli* 08 and 09, and *Klebsiella* O3 and O5 serotypes (all of these O polysaccharides are mannose homopolymers and the structures of the repeating unit of *E. coli* 09 and *Klebsiella* O3 are identical) showed that a central region specific for *E. coli* 09 and *Klebsiella* O3 is flanked by two regions common to all four. Complementation experiments using strains with known defects and specific tests for the enzymic activity showed that the 50 and 55 kDa polypeptides, encoded by the common region, are phosphomannomutase (PMM) and GDP-mannose pyrophosphorylase (GMP), respectively. Nucleotide sequencing of the region revealed the presence of two genes, *rfbK* and *rfbM*, analogous to the corresponding genes of *Salmonella typhimurium*. In *E. coli* 09, *rfbK* and *rfbM* encode proteins of 460 amino acids (50,809 Da) and 471 amino acids (52,789 Da). The amino acid sequence of GMP was conserved in *RfbMs* of *E. coli* O7 and *Salmonella* groups B, C1 and C2, *CpsB* of *S. typhimurium*, *AlgA* of *Pseudomonas aeruginosa*, and *XanB* of *Xanthomonas campestris*. The phylogenetic trees of PMM and GMP were different in topology and in the evolutionary distances from ancestors.

3/3,AB/24 (Item 24 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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08223113 95244392

Genetic analysis of the acetan biosynthetic pathway in *Acetobacter xylinum*.

Griffin AM; Morris VJ; Gasson MJ

Institute of Food Research, Norwich Laboratory, Colney, UK.

International journal of biological macromolecules (ENGLAND) Dec 1994, 16 (6) p287-9, ISSN 0141-8130 Journal Code: AY6

Languages: ENGLISH

Document type: JOURNAL ARTICLE

We have identified, cloned and sequenced an 8422 base pair fragment of *Acetobacter xylinum* genomic DNA containing part of the acetan biosynthetic gene cluster. Computer analysis of the nucleotide sequence data generated revealed the presence of six open reading frames. Comparison of the translated sequences of putative genes to the amino acid sequences of genes from other organisms was used to assign functions to the *aceA*, *aceC* and *manB* genes. These genes were predicted to encode a UDP-glycosyl transferase, a GDP-mannosyl transferase and a phosphomannose isomerase/GDP-mannose pyrophosphorylase, respectively.

3/3,AB/25 (Item 2 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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08197893 94148934

Identification of amino acid residues involved in the activity of phosphomannose isomerase-guanosine 5'-diphospho-D-mannose pyrophosphorylase. A bifunctional enzyme in the alginate biosynthetic pathway of *Pseudomonas aeruginosa*.

May TB; Shinabarger D; Boyd A; Chakrabarty AM

Department of Microbiology and Immunology, University of Illinois College of Medicine, Chicago 60612.

Journal of biological chemistry (UNITED STATES) Feb 18 1994, 269 (7) p4872-7, ISSN 0021-9258 Journal Code: HIV

Contract/Grant No.: AI-16790-13, AI, NIAID

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Phosphomannose isomerase-guanosine 5'-diphospho-D-mannose pyrophosphorylase (PMI-GMP), which is encoded by the *algA* gene, catalyzes two noncontiguous steps in the alginate biosynthetic pathway of *Pseudomonas aeruginosa*; the isomerization of D-fructose 6-phosphate to D-mannose

6-phosphate and the synthesis of GDP-D-mannose and PPi from GTP and D-mannose 1-phosphate. Amino acids that are required for the GMP enzyme activity were identified through site-directed mutagenesis of the *algA* gene. Mutation of Lys-175 to arginine, glutamine, or glutamate produced an enzyme whose *Km* for D-mannose 1-phosphate was 470-3,200-fold greater than that measured for the wild type enzyme. In addition, these mutant enzymes had a lower *Vmax* for the GMP activity as compared with the wild type PMI-GMP. These results indicate that Lys-175 is primarily involved in the binding of the substrate D-mannose 1-phosphate, although it is likely that other residues are required for the specificity of binding. Mutation of Arg-19 to glutamine, histidine, or leucine resulted in a 2-fold lower *Vmax* for the GMP enzyme activity and a 4-7-fold increase in the *Km* for GTP as compared with the wild type enzyme. Thus, it appears that Arg-19 functions in the binding of GTP. In addition, chymotryptic digestion of PMI-GMP showed that the carboxyl terminus is critical for PMI activity but not for GMP activity. Taken together, these results support the hypothesis that the bifunctional PMI-GMP protein is composed of two independent enzymatic domains.

3/3,AB/26 (Item 26 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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08182776 94252978

Cloning and analysis of duplicated *rfbM* and *rfbK* genes involved in the formation of GDP-mannose in *Escherichia coli* O9:K30 and participation of *rfb* genes in the synthesis of the group I K30 capsular polysaccharide.

Jayaratne P; Bronner D; MacLachlan PR; Dodgson C; Kido N; Whitfield C

Department of Microbiology, University of Guelph, Ontario, Canada.

Journal of bacteriology (UNITED STATES) Jun 1994, 176 (11) p3126-39, ISSN 0021-9193 Journal Code: HH3

Languages: ENGLISH

Document type: JOURNAL ARTICLE

The *rfbO9* gene cluster, which is responsible for the synthesis of the lipopolysaccharide O9 antigen, was cloned from *Escherichia coli* O9:K30. The *gnd* gene, encoding 6-phosphogluconate dehydrogenase, was identified adjacent to the *rfbO9* cluster, and by DNA sequence analysis the gene order *gnd-rfbM-rfbK* was established. This order differs from that described for other members of the family Enterobacteriaceae. Nucleotide sequence analysis was used to identify the *rfbK* and *rfbM* genes, encoding phosphomannomutase and GDP-mannose pyrophosphorylase,

respectively. In members of the family Enterobacteriaceae, these enzymes act sequentially to form GDP-mannose, which serves as the activated sugar nucleotide precursor for mannose residues in cell surface polysaccharides. In the *E. coli* O9:K30 strain, a duplicated *rfbM2-rfbK2* region was detected approximately 3 kbp downstream of *rfbM1-rfbK1* and adjacent to the remaining genes of the *rfbO9* cluster. The *rfbM* isogenes differed in upstream flanking DNA but were otherwise highly conserved. In contrast, the *rfbK* isogenes differed in downstream flanking DNA and in 3'-terminal regions, resulting in slight differences in the sizes of the predicted RfbK proteins. RfbMO9 and RfbKO9 are most closely related to CpsB and CpsG, respectively. These are isozymes of GDP-mannose pyrophosphorylase and phosphomannomutase, respectively, which are thought to be involved in the biosynthesis of the slime polysaccharide colanic acid in *E. coli* K-12 and *Salmonella enterica* serovar Typhimurium. An *E. coli* O-:K30 mutant, strain CWG44, lacks *rfbM2-rfbK2* and has adjacent essential *rfbO9* sequences deleted. The remaining chromosomal genes are therefore sufficient for GDP-mannose formation and K30 capsular polysaccharide synthesis. A mutant of *E. coli* CWG44, strain CWG152, was found to lack GDP-mannose pyrophosphorylase and lost the ability to synthesize K30 capsular polysaccharide. Wild-type capsular polysaccharide could be restored in CWG152, by transformation with plasmids containing either *rfbM1* or *rfbM2*. Introduction of a complete *rfbO9* gene cluster into CWG152 restored synthesis of both O9 and K30 polysaccharides. Consequently, *rfbM* is sufficient for the biosynthesis of GDP-mannose for both O antigen and capsular polysaccharide *E. coli* O9:K30. Analysis of a collection of serotype O8 and O9 isolates by Southern hybridization and PCR amplification experiments demonstrated extensive polymorphism in the *rfbM-rfbK* region.

3/3,AB/27 (Item 27 from file: 155)

DIALOG(R) File 155:MEDLINE(R)

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07816874 93252670

Manipulation of *Pseudomonas aeruginosa* alginate pathway by varying the level of biosynthetic enzymes and growth temperature.

Leitao JH; Sa-Correia I

Laboratorio de Engenharia Bioquimica, Instituto Superior Tecnico, Lisboa, Portugal.

Journal of applied bacteriology (ENGLAND) Apr 1993, 74 (4) p452-9, ISSN 0021-8847 Journal Code: HDJ

Languages: ENGLISH

Document type: JOURNAL ARTICLE

The manipulation of the alginate pathway in two *Pseudomonas aeruginosa* mucoid variants was attempted at growth temperatures within the range 20 degrees C-40 degrees C. This was carried out by increasing the level of either phosphomannose isomerase (PMI) and GDP-mannose pyrophosphorylase (GMP) or GDP-mannose dehydrogenase (GMD) encoded by *algA* or *algD* respectively, present in recombinant plasmids derived from the controlled expression vector pMMB24. The specific growth rate of cells expressing either *algA* or *algD* genes from recombinant plasmids was lower than that of cells harbouring the cloning vector only. Stimulation of alginate synthesis was observed when the expression of the alginate genes was low, in the absence of isopropyl-beta-D-thiogalactopyranoside (IPTG) induction. The further increase of the level of alginate enzymes in induced cells, without the simultaneous increase of other limiting steps, had no positive effect on the strictly regulated alginate pathway. Temperature profiles for alginate synthesis were modified reflecting changes in rate limiting steps. Limitations on the polymerization ability and the competition between cell growth and alginate synthesis were possibly involved in the modification of the temperature profiles for alginate production, or in the decrease of the molecular weight of polymers produced by recombinants under conditions that led to highly active alginate synthesis. The acetyl content of alginates

produced by the recipients was higher than that of the biopolymer controls, possibly due to the higher acetyl-CoA availability in slower growing cells.

3/3,AB/28 (Item 28 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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07815580 93232764

Oxygen-dependent alginate synthesis and enzymes in *Pseudomonas aeruginosa*.

Leitao JH; Sa-Correia I
Laboratorio de Engenharia Bioquimica, Instituto Superior Tecnico, Lisboa, Portugal.

Journal of general microbiology (ENGLAND) Mar 1993, 139 (Pt 3) p441-5
ISSN 0022-1287 Journal Code: I87

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Alginate production by the highly alginate-producing *Pseudomonas aeruginosa* 8821M was maximal at a dissolved oxygen tension (DOT) of 5% of air saturation. Lower DOT limited growth and alginate synthesis. At higher DOT values up to 70% of air saturation, the specific alginate production rate decreased. Nevertheless, the molecular mass of the alginate increased at higher aerations, as indicated by the viscosity of solutions of the isolated biopolymer. The specific activity of the four enzymes leading to GDP-mannuronic acid formation, phosphomannose isomerase (PMI), phosphomannomutase (PMM), GDP-mannose pyrophosphorylase (GMP) and GDP-mannose dehydrogenase (GMD), increased with DOT of up to 25%. At higher DOT, however, only GMP and GMD maintained their maximum values. Changes observed at high oxygen concentrations in the relative activities of PMI and GMP, which are activities of the same bifunctional protein, were attributed to the much higher sensitivity of PMI activity to irreversible oxidative inactivation. The less pronounced decrease of PMM activity at high DOT correlated with an intermediate sensitivity to oxidative inactivation, but could also be related to sequential induction of PMM by the product of the PMI reaction. Thus, oxygen-dependence of alginate synthesis was at least partially the effect of DOT on GDP-mannuronic acid formation. Optimal aerations for maximal alginate production (DOT = 5-10%) were below the aeration level (70%) that led to the highest viscosity. These results suggest that, like GMD, polymerization activity is not very sensitive to oxidative inactivation and they are consistent with the hypothesis that polymerization is dependent on GMD activity, or is regulated in a similar way.

3/3,AB/29 (Item 29 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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07795319 93106949

Identification, expression, and DNA sequence of the GDP-mannose biosynthesis genes encoded by the O7 rfb gene cluster of strain VW187 (*Escherichia coli* O7:K1).

Marolda CL; Valvano MA
Department of Microbiology and Immunology, University of Western Ontario, London, Canada.

Journal of bacteriology (UNITED STATES) Jan 1993, 175 (1) p148-58,
ISSN 0021-9193 Journal Code: HH3

Languages: ENGLISH

Document type: JOURNAL ARTICLE

The O7-specific lipopolysaccharide (LPS) in strains of *Escherichia coli* consists of a repeating unit made of galactose, mannose, rhamnose, 4-acetamido-2,6-dideoxyglucose, and N-acetylglucosamine. We have recently

cloned and characterized genetically the O7-specific LPS biosynthesis region (rfbEcO7) of the *E. coli* O7:K1 strain VW18 (C. L. Marolda, J. Welsh, L. Dafoe, and M. A. Valvano, *J. Bacteriol.* 172:3590-3599, 1990). In this study, we localized the *gnd* gene encoding gluconate-6-phosphate dehydrogenase at one end of the rfbEcO7 gene cluster and sequenced that end of the cluster. Three open reading frames (ORF) encoding polypeptides of 275, 464, and 453 amino acids were identified upstream of *gndEcO7*, all transcribed toward the *gnd* gene. ORF275 had 45% similarity at the protein level with ORF16.5, which occupies a similar position in the *Salmonella enterica* LT2 rfb region, and presumably encodes a nucleotide sugar transferase. The polypeptides encoded by ORFs 464 and 453 were expressed under the control of the *ptac* promoter and visualized in Coomassie blue-stained sodium dodecyl sulfate-polyacrylamide gels and by maxicell analysis. ORF464 expressed GDP-mannose pyrophosphorylase and ORF453 encoded a phosphomannomutase, the enzymes for the biosynthesis pathway of GDP-mannose, one of the nucleotide sugar precursors for the formation of the O7 repeating unit. They were designated rfbMEcO7 and rfbKEcO7, respectively. The RfbMEcO7 polypeptide was homologous to the corresponding protein in *S. enterica* LT2, XanB of *Xanthomonas campestris*, and AlgA of *Pseudomonas aeruginosa*, all GDP-mannose pyrophosphorylases. RfbKEcO7 was very similar to CpsG of *S. enterica* LT2, an enzyme presumably involved in the biosynthesis of the capsular polysaccharide colanic acid, but quite different from the corresponding RfbK protein of *S. enterica* LT2.

3/3,AB/30 (Item 30 from file: 155)
 DIALOG(R) File 155:MEDLINE(R)
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07785240 93352609

GDP-mannose pyrophosphorylase . Purification to homogeneity, properties, and utilization to prepare photoaffinity analogs. Szumilo T; Drake RR; York JL; Elbein AD
 Department of Biochemistry and Molecular Biology, University of Arkansas for Medical Sciences, Little Rock 72205-7199.
 Journal of biological chemistry (UNITED STATES) Aug 25 1993, 268 (24) p17943-50, ISSN 0021-9258 Journal Code: HIV
 Contract/Grant No.: DK-21800, DK, NIDDK
 Languages: ENGLISH
 Document type: JOURNAL ARTICLE

Pig liver GDP-mannose pyrophosphorylase was purified
 5,000-fold to apparent homogeneity using standard techniques. The native enzyme showed a single band on gels of about 450 kDa and two subunits of 43 and 37 kDa on sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The 37-kDa (beta-) subunit had only methionine at its amino terminus and a surprisingly hydrophobic sequence: Met-Lys-Ala-Leu-Ile-Leu-Val-Gly-Gly-Tyr-Gly-Thr-Arg-Leu-Arg-Pro-Leu-Thr-Leu-Ser-Ile-Pro-Lys. The 43-kDa (alpha-) subunit was blocked at the amino terminus, but a 29-kDa CNBr fragment had the following sequence: Leu-Asp-Ala-His-Arg-His-Arg-Pro-His-Pro-Phe-Leu-Leu-. Substrate specificity studies done in the direction of formation of nucleoside triphosphate and sugar-1-P indicated that the enzyme was most effective with GDP -glucose as substrate (100%) followed by IDP-mannose (72%) and then GDP-mannose (61%). That GDP-mannose and GDP-glucose activities were indeed catalyzed by the same enzyme was indicated by the following. (i) Various studies indicated that the enzyme was homogeneous. (ii) A staining procedure for production of GTP stained the same single band on native gels when either GDP-mannose or GDP-glucose was the substrate. (iii). GDP-mannose inhibited the utilization of GDP -glucose by the enzyme, and vice versa. When 8-azido-[32P]GTP was incubated with native enzyme and exposed to UV light, both the 43-kDa and the 37-kDa subunits became labeled, although the 37-kDa subunit reacted more strongly. On the other hand, 8-azido-GDP-[32P]mannose only photolabeled the 43-kDa band. Most importantly, the purified enzyme can be utilized to

produce 8-azido-[32P] mannose or 8-azido-[32P]GDP
glucose.

3/3,AB/31 (Item 31 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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07376819 92104962

Genetics of xanthan production in *Xanthomonas campestris*: the xanA and xanB genes are involved in UDP-glucose and GDP-mannose biosynthesis.

Koplin R; Arnold W; Hotte B; Simon R; Wang G; Puhler A
Lehrstuhl für Genetik, Universität Bielefeld, Germany.
Journal of bacteriology (UNITED STATES) Jan 1992, 174 (1) p191-9,
ISSN 0021-9193 Journal Code: HH3
Languages: ENGLISH

Document type: JOURNAL ARTICLE

The nucleotide sequence of a 3.4-kb EcoRI-PstI DNA fragment of *Xanthomonas campestris* pv. *campestris* revealed two open reading frames, which were designated xanA and xanB. The genes xanA and xanB encode proteins of 448 amino acids (molecular weight of 48,919) and 466 amino acids (molecular weight of 50,873), respectively. These genes were identified by analyzing insertion mutants which were known to be involved in xanthan production. Specific tests for the activities of enzymes involved in the biosynthesis of UDP-glucose and GDP-mannose indicated that the xanA gene product was involved in the biosynthesis of both glucose 1-phosphate and mannose 1-phosphate. The deduced amino acid sequence of xanB showed a significant degree of homology (59%) to the phosphomannose isomerase of *Pseudomonas aeruginosa*, a key enzyme in the biosynthesis of alginate. Moreover, biochemical analysis and complementation experiments with the *Escherichia coli* manA fragment revealed that xanB encoded a bifunctional enzyme, phosphomannose isomerase-GDP-mannose pyrophosphorylase.

3/3,AB/32 (Item 32 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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07353683 91115815

Purification and characterization of phosphomannose isomerase-guanosine diphospho-D-mannose pyrophosphorylase. A bifunctional enzyme in the alginate biosynthetic pathway of *Pseudomonas aeruginosa*.

Shinabarger D; Berry A; May TB; Rothmel R; Fialho A; Chakrabarty AM
Department of Microbiology and Immunology, University of Illinois College of Medicine, Chicago 60612.

Journal of biological chemistry (UNITED STATES) Feb 5 1991, 266 (4) p2080-8, ISSN 0021-9258 Journal Code: HIV

Contract/Grant No.: AI-16790-12, AI, NIAID; F32AI07890, AI, NIAID

Languages: ENGLISH

Document type: JOURNAL ARTICLE

We report here the purification and characterization of phosphomannose isomerase-guanosine 5'-diphospho-D-mannose pyrophosphorylase, a bifunctional enzyme (PMI-GMP) which catalyzes both the phosphomannose isomerase (PMI) and guanosine 5'-diphospho-D-mannose pyrophosphorylase (GMP) reactions of the *Pseudomonas aeruginosa* alginate biosynthetic pathway. The PMI and GMP activities co-eluted in the same protein peak through successive fractionation on hydrophobic interaction, ion exchange, and gel filtration chromatography. The purified enzyme migrated as a 56,000 molecular weight protein on sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and the native protein migrated as a monomer of 54,000 molecular weight upon gel filtration chromatography. The apparent K_m for D-mannose 6-phosphate was 3.03 mM, and the V_{max} was 830 nmol/min/mg of enzyme. For the GMP forward reaction, apparent K_m

values of 20.5 microM and 29.5 microM for D-mannose 1-phosphate and GTP, respectively, were obtained from double reciprocal plots. The GMP forward reaction Vmax (5,680 nmol/min/mg of enzyme) was comparable to the reverse reaction Vmax (5,170 nmol/min/mg of enzyme), and the apparent Km for GDP-D-mannose was determined to be 14.2 microM. Both reactions required Mg2+ activation, but the PMI reaction rate was 4-fold higher with Co2+ as the activator. PMI (but not GMP) activity was sensitive to dithiothreitol, indicating the involvement of disulfide bonds to form a protein structure capable of PMI activity. DNA sequencing of a cloned mutant algA gene from *P. aeruginosa* revealed that a point mutation at nucleotide 961 greatly decreased the levels of both PMI and GMP in a crude extract.

3/3,AB/33 (Item 33 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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07327199 93018988

Sequence and structural analysis of the rfb (O antigen) gene cluster from a group C1 *Salmonella enterica* strain.

Lee SJ; Romana LK; Reeves PR

Department of Microbiology, University of Sydney, Australia.

Journal of general microbiology (ENGLAND) Sep 1992, 138 (Pt 9)
p1843-55, ISSN 0022-1287 Journal Code: I87

Languages: ENGLISH

Document type: JOURNAL ARTICLE

The rfb (O antigen) gene cluster of a group C1 *Salmonella enterica* strain was sequenced; it comprised seven open reading frames which precisely replaced the 16 open reading frames of a group B strain. Two genes of the mannose biosynthetic pathway were present: rfbK (phosphomannomutase) had a G+C content of 0.61 and had only 40% identity to rfbK of group B but was very similar to cpsG of the capsular polysaccharide pathway with 96% identity, whereas rfbM [guanosine diphosphomannose (GDP-Man) pyrophosphorylase] had a G+C content of 0.39. Other genes had G+C contents ranging from 0.24 to 0.28. rfbM(C1) and rfbM(B) had 60% identity, which is much less than expected within a species, but nonetheless indicates a much more recent common ancestor than for rfbK. The other genes showed much lower or no similarity to rfb genes of other *S. enterica* strains. It appears that the gene cluster evolved outside of *Salmonella* in a species with low G+C content: the rfbM gene presumably derives from that period whereas the rfbK gene appears to have arisen after transfer of the cluster to *S. enterica* by duplication of the *S. enterica* cpsG gene, presumably replacing an rfbK gene of low G+C content.

3/3,AB/34 (Item 34 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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06198212 87250293

Alginate biosynthetic enzymes in mucoid and nonmucoid *Pseudomonas aeruginosa*: overproduction of phosphomannose isomerase, phosphomannomutase, and GDP-mannose pyrophosphorylase by overexpression of the phosphomannose isomerase (pmi) gene.

Sa-Correia I; Darzins A; Wang SK; Berry A; Chakrabarty AM

Journal of bacteriology (UNITED STATES) Jul 1987, 169 (7) p3224-31,
ISSN 0021-9193 Journal Code: HH3

Contract/Grant No.: AI-16790-08, AI, NIAID

Languages: ENGLISH

Document type: JOURNAL ARTICLE

The specific activities of phosphomannose isomerase (PMI), phosphomannomutase (PMM), GDP-mannose pyrophosphorylase (GMP), and GDP-mannose dehydrogenase (GMD) were compared in a mucoid cystic fibrosis isolate of *Pseudomonas aeruginosa* and in two

spontaneous nonmucoid revertants. In both revertants some or all of the alginate biosynthetic enzymes we examined appeared to be repressed, indicating that the loss of the mucoid phenotype may be a result of decreased formation of sugar-nucleotide precursors. The introduction and overexpression of the cloned *P. aeruginosa* phosphomannose isomerase (pmi) gene in both mucoid and nonmucoid strains led not only to the appearance of PMI levels in cell extracts several times higher than those present in the wild-type mucoid strain, but also in higher PMM and GMP specific activities. In extracts of both strains, however, the specific activity of GMD did not change as a result of pmi overexpression. In contrast, the introduction of the cloned *Escherichia coli* manA (pmi) gene in *P. aeruginosa* caused an increase in only PMI and PMM activities, having no effect on the level of GMP. This suggests that an increase in PMI activity alone does not induce high GMP activity in *P. aeruginosa*. The heterologous overexpression of the *P. aeruginosa* pmi gene in the *E. coli* manA mutant CD1 led to the appearance in cell extracts of not only PMI activity but also GMP activity, both of which are normally undetectable in extracts of CD1. We discuss the implications of these results and propose a mechanism by which overexpression of the *P. aeruginosa* pmi gene can cause an elevation in both PMM and GMP activities.

3/3,AB/35 (Item 35 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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05942171 88171439

Characterization of the *Pseudomonas aeruginosa* alginate (alg) gene region II.

Wang SK; Sa'-Correia I; Darzins A; Chakrabarty AM

Department of Microbiology and Immunology, University of Illinois Health Sciences Center, Chicago 60612.

Journal of general microbiology (ENGLAND) Aug 1987, 133 (Pt 8)
p2303-14, ISSN 0022-1287 Journal Code: I87

Contract/Grant No.: AI 16790-08, AI, NIAID

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Pseudomonas aeruginosa region II alginate genes are involved in the biosynthesis of the uronic acid containing exopolysaccharide, alginate. We have subcloned and overexpressed various DNA fragments contained within region II in an attempt to further characterize and more precisely localize the genes involved in alginate production. Overexpression of the genes controlling alginate biosynthesis within region II was accomplished by placing various cloned restriction fragments under the transcriptional control of the hybrid trp-lac (tac) promoter, and plasmid encoded proteins were examined in a maxicell expression system. We correlated various region II plasmid constructions with the ability to complement specific alginate negative (alg) mutants and code for polypeptides. Several proteins suspected of being involved in alginate production were encoded by sequences within region II. The results of this study further reveal that the transcriptional orientation of the alg loci within region II appears to be in the direction from *argF* to *pmi*. The specific activities of phosphomannomutase (PMM) and GDP-mannose pyrophosphorylase (GMP), two enzymes involved in the formation of the alginate precursor GDP-mannuronic acid, were measured in region II alg mutants and in cells overexpressing cloned segments from region II. Based on these enzyme measurements, we conclude that the remaining region II alg genes do not encode either PMM or GMP. These results support the suggestion that the remaining alg genes in region II are likely to be involved in post GDP-mannuronic acid processing events such as mannuronic acid transport, polymerization, secretion, epimerization and acetylation.

3/3,AB/36 (Item 36 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

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03158777 75208982

Regulation of the synthesis of nucleoside diphosphate sugars in reticulo-endothelial tissues.

Mendicino J; Rao AK

European journal of biochemistry (GERMANY, WEST) Feb 21 1975, 51 (2) p547-56, ISSN 0014-2956 Journal Code: EMZ

Languages: ENGLISH

Document type: JOURNAL ARTICLE

The kinetic and regulatory properties of enzymes involved in the biosynthesis of UDP-D-galactose, UDP-N-acetylglucosamine. **GDP** -alpha-D-mannose and **GDP** -beta-L-fucose from D-glucose 6-phosphate in various reticulo-endothelial tissues was studied. The tissues examined include bovine liver, thyroid, spleen, salivary gland, lung, intestine and mesenteric; pulmonary, portal and sub-maxillary lymphnodes. The maximum rates of specific enzymes in these pathways which were slow enough to be rate-limiting in the formation of glycoproteins in these tissues was determined. UDP-D-galactose 4-epimerase was consistently the rate-limiting reaction in the conversion of -d-glucose 6-phosphate to UDP-D-galactose in all of the tissues examined. The series of reactions leading to the formation of **GDP**-alpha-D-mannose and **GDP** -beta-L-fucose were limited by the activity of **GDP**-alpha-D-mannose **pyrophosphorylase** and **GDP**-alpha-D-mannose oxidoreductase, respectively. The formation of UDP-N-acetylglucosamine was limited by the rate of the amination reaction which converts -d-fructose 6-phosphate to D-glucosamine 6-phosphate in the presence of glutamine. Several of these rate-limiting enzymes were partially purified from mesenteric lymph node extracts, and their regulatory properties were examined. **GDP**-alpha-D-mannose was found to be a competitive inhibitor of **GDP**-alpha-D-mannose **pyrophosphorylase**. The apparent Km for GTP was 0.06 mM and the Ki for **GDP**-alpha-D-mannose was 0.03 mM. The concentrations of GTP and **GDP**-alpha-D-mannose in lymph node extracts were determined to be 0.095 and 0.012 mumol per g, respectively. UDP-N-acetylglucosamine and UDP-D-glucose inhibited D-fructose 6-phosphate amidotransferase in a manner competitive with D-fructose 6-phosphate. The Km for fructose 6-phosphate was 0.3 mM, while the Ki for UDP-D-glucose and UDP-N-acetylglucosamine were determined to be 0.4 mM and 0.045 mM, respectively. The concentrations of these metabolites in lymph node tissue were: UDP-D-glucose, 0.42; UDP-N-acetylglucosamine 0.095; and D-fructose 6-phosphate, 0.073 mumol per g wet weight of tissue. The results obtained in these studies show that specific rate-limiting enzymes in the pathways for the biosynthesis of nucleoside diphosphate sugars in reticulo-endothelial tissues may be subject to cumulative feedback inhibition by the nucleoside diphosphate sugars which are the final products of these systems and the initial precursors of the oligosaccharide units of glycoproteins in these tissues.

3/3,AB/37 (Item 37 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

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3/3,AB/38 (Item 1 from file: 5)

DIALOG(R)File 5:Biosis Previews(R)

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11515040 BIOSIS NO.: 199800296372

The cofactor Mg+2!+-a key switch for effective continuous enzymatic production of GDEP-mannose using recombinant **GDP**-mannose **pyrophosphorylase**.

AUTHOR: Fey Sven; Elling Loothar; Kragl Udo(a)

AUTHOR ADDRESS: (a)Inst. Biotechnologie, Forschungszentrum Juelich GmbH,

D-52425 Juelich**Germany
JOURNAL: Carbohydrate Research 305 (3-4):p475-481 Dec. 1997
ISSN: 0008-6215
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The aim of the presented work is the chemoenzymatic synthesis of **GDP-mannose (GDP-Man)**. **Mannose-1-phosphate (Man-1-P)** is synthesised by a very convenient chemical method and is activated with GTP and recombinant **GDP-mannose pyrophosphorylase (GDP-Man PP)** to give **GDP-Man**. The productivity of the process is improved using reaction engineering techniques. Detailed kinetic studies, modelling of the reaction and simulations of different reaction systems revealed that besides a strong product inhibition the cofactor Mg^{2+} is a key switch for effective enzymatic synthesis. Using a two-stage cascade of enzyme membrane reactors, **GDP-Man** could be produced continuously with a space-time yield of 28 g L⁻¹ d⁻¹ and an enzyme consumption of 0.9 U g⁻¹, which means a six-fold improvement related to batch synthesis.

1997

3/3,AB/39 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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10841007 BIOSIS NO.: 199799462152
Effects of growth-inhibitory concentrations of copper on alginate biosynthesis in highly mucoid *Pseudomonas aeruginosa*.
AUTHOR: Leitao Jorge H; Sa-Correia Isabel(a)
AUTHOR ADDRESS: (a)Lab. Engenharia Bioquimica, Cent. Engenharia Biol. Quimica, Inst. Superior Tecnico, Av. Rovisco **Portugal
JOURNAL: Microbiology (Reading) 143 (2):p481-488 1997
ISSN: 1350-0872
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Alginate production and degree of polymerization were affected when the highly mucoid *Pseudomonas aeruginosa* 8821 M was grown with growth-inhibitory concentrations of Cu-2+ (supplied as CuCl₂; 1-5 mM). The inhibition of alginate biosynthesis was consistent with the decreased activity in Cu-2+-stressed cells of phosphomannose isomerase/**GDP-mannose pyrophosphorylase** (encoded by algA), phosphomannomutase (encoded by algC) and **GDP-mannose dehydrogenase** (encoded by algD). However, in cells grown with concentrations of CuCl₂ below 2 mM, the steady-state mRNA levels from algA, algC, algD and from the regulatory gene algR1 increased moderately. This observation is consistent with the suggested linkage between the control of alginate gene expression and the global regulation involved in the oxidative stress response. At highly inhibitory concentrations the levels of the four alginate gene transcripts decreased from maximal values. The bell-shaped curves, representing the effect of Cu-2+ concentration on mRNA levels from the four alginate genes, exhibited similar patterns but did not concur. The decrease of the specific activity of enzymes necessary for **GDP-mannuronic acid** synthesis in Cu-2+-grown cells was correlated with changes in gene expression, with the inhibitory effect of Cu-2+ on enzyme activities and with Cu-2+-induced oxidative inactivation of enzymes, especially the particularly sensitive phosphomannose isomerase activity.

1997

3/3,AB/40 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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10107337 BIOSIS NO.: 199698562255

Sequence and analysis of the O antigen gene (rfb) cluster of *Escherichia coli* O111.

AUTHOR: Bastin David A; Reeves Peter R(a)

AUTHOR ADDRESS: (a)Dep. Microbiol., G08, Univ. Sydney, Sydney, NSW 2006**
Australia

JOURNAL: Gene (Amsterdam) 164 (1):p17-23 1995

ISSN: 0378-1119

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: The O antigens found in *Salmonella enterica* (Se) and *Escherichia coli* (Ec) show a great deal of diversity, and only three structures are known to be common to both genera. Two of them contain the 3,6-dideoxyhexose colitose, not found in other serogroups of the two species. The first of these is common to Ec O111 and Se O:35 (sv Adelaide); the other is found in both Ec O55 and Se O:50 (sv Greenside). The genes specific for the synthesis of O antigen are generally located in the rfb gene cluster at map position 45 min in Ec and 42 min in Se. The rfb (O antigen) gene cluster of an Ec O111 strain M92 had been cloned earlier and hybridisation analysis suggested that the rfb clusters of Ec M92 and a Se sv Adelaide strain had been acquired separately by the two species since their divergence. We have now sequenced part of the rfb cluster from Ec M92. We identify two genes of the **GDP-colitose** pathway, rfbM and rfbK, and show that several other ORFs have similarity to the rfb and cps (capsular polysaccharide) genes. Downstream of this block of genes is an ORF which encodes a protein with predicted transmembrane segments which is presumed to correspond to the rfbX gene. The % G + C values of the Ec M92 rfb sequence are extremely low, indicating that the rfb evolved in a low % G + C species of bacteria before transfer into Ec.

1995

3/3,AB/41 (Item 4 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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09579078 BIOSIS NO.: 199598033996

Studies on some enzymes of alginic acid biosynthesis in mucoid and nonmucoid *Azotobacter chroococcum* strains.

AUTHOR: Pecina A; Paneque A(a)

AUTHOR ADDRESS: (a)Inst. Bioquim. Veg. y Fotosintesis, Univ.

Sevilla-Consejo Superior Investigaciones Cientificas, **Spain

JOURNAL: Applied Biochemistry and Biotechnology 49 (1):p51-58 1994

ISSN: 0273-2289

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Measurements of enzymes involved in alginate biosynthesis were straightforward in mucoid (alginate-positive) *Azotobacter chroococcum* ATCC 4412 crude extracts. At the stationary growth phase, where the production of the exopolysaccharide was greatest, the enzymes phosphomannose isomerase and **GDP-mannose pyrophosphorylase** increased markedly, whereas phosphomannomutase and **GDP-mannose** dehydrogenase kept the high activity levels measured in the acceleration growth phase. In nonmucoid (alginatenegative) *A. chroococcum* and *A. vinelandii* strains, the

activities of phosphomannose isomerase and **GDP-mannose pyrophosphorylase** were rather low or, in some cases, undetectable. Except in *A. chroococcum* MCD1, which exhibited a low activity, phosphomannomutase was high in the nonmucoid *Azotobacter* strains, and **GDP-mannose** dehydrogenase reached a significant activity level in two out of four nonmucoid strains tested. The results suggest that derepression of phosphomannose isomerase and **GDP-mannose pyrophosphorylase** is a *sine qua non* condition for alginate formation by *A. chroococcum*.

1994

3/3,AB/42 (Item 5 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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09304157 BIOSIS NO.: 199497312527

Cloning and analysis of duplicated *rfbM* and *rfbK* genes involved in the formation of **GDP-mannose** in *Escherichia coli* O9:K30 and participation of *rbf* genes in the synthesis of the group I K30 capsular polysaccharide.

AUTHOR: Jayaratne Padman; Bronner Dorothea; MacLachlan P Ronald; Dodgson Christine; Kido Nobuo; Whitfield Chris(a)

AUTHOR ADDRESS: (a)Dep. Microbiol. Univ. Guelph, Guelph, ON N1G 2W1**Canada

JOURNAL: Journal of Bacteriology 176 (11):p3126-3139 1994

ISSN: 0021-9193

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: The *rfb*-O9 gene cluster, which is responsible for the synthesis of the lipopolysaccharide O9 antigen, was cloned from *Escherichia coli* O9:K30. The *gnd* gene, encoding 6-phosphogluconate dehydrogenase, was identified adjacent to the *rfb*-O9 cluster, and by DNA sequence analysis the gene order *gnd*-*rfbM*-*rfbK* was established. This order differs from that described for other members of the family Enterobacteriaceae. Nucleotide sequence analysis was used to identify the *rfbK* and *rfbM* genes, encoding phosphomannomutase and **GDP-mannose pyrophosphorylase**, respectively. In members of the family Enterobacteriaceae, these enzymes act sequentially to form **GDP-mannose**, which serves as the activated sugar nucleotide precursor for **mannose** residues in cell surface polysaccharides. In the *E. coli* O9:K30 strain, a duplicated *rfbM*-2-*rfbK*-2 region was detected approximately 3 kbp downstream of *rfbM*-1-*rfbK*-1 and adjacent to the remaining genes of the *rfb*-O9 cluster. The *rfbM* isogenes differed in upstream flanking DNA but were otherwise highly conserved. In contrast, the *rfbK* isogenes differed in downstream flanking DNA and in 3'-terminal regions, resulting in slight differences in the sizes of the predicted *RfbK* proteins. *RfbM*-O9 and *RfbK*-O9 are most closely related to *CpsB* and *CpsG*, respectively. These are isozymes of **GDP-mannose pyrophosphorylase** and phosphomannomutase, respectively, which are thought to be involved in the biosynthesis of the slime polysaccharide colanic acid in *E. coli* K-12 and *Salmonella enterica* serovar Typhimurium. An *E. coli* O-:K30 mutant, strain CWG44, lacks *rfbM*-2-*rfbK*-2 and has adjacent essential *rfb*-O9 sequences deleted. The remaining chromosomal genes are therefore sufficient for **GDP-mannose** formation and K30 capsular polysaccharide synthesis. A mutant of *E. coli* CWG44, strain CWG152, was found to lack **GDP-mannose pyrophosphorylase** and lost the ability to synthesize K30 capsular polysaccharide. Wild-type capsular polysaccharide could be restored in CWG152, by transformation with plasmids containing either *rfbM*-1 or *rfbM*-2. Introduction of a complete *rfb*-O9 gene cluster into CWG152 restored synthesis of both O9 and K30 polysaccharides. Consequently, *rfbM* is sufficient for the biosynthesis of **GDP-mannose** for both O antigen and capsular

polysaccharide in *E. coli* O9:K30. Analysis of a collection of serotype O8 and O9 isolates by Southern hybridization and PCR amplification experiments demonstrated extensive polymorphism in the *rfbM-rfbK* region.

1994

3/3,AB/43 (Item 6 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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08846173 BIOSIS NO.: 199395135524

Identification, genetic and biochemical analysis of genes involved in synthesis of sugar nucleotide precursors of xanthan gum.

AUTHOR: Harding Nancy E; Raffo Susana; Raimondi Alejandra; Cleary Joseph M; Telpi Luis(a)

AUTHOR ADDRESS: (a)Inst. Investigaciones Bioquimicas 'Fundacion Campomar', Fac. Ciencias Exactas y Naturales, CONIC**Argentina

JOURNAL: Journal of General Microbiology 139 (3):p447-457 1993

ISSN: 0022-1287

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: A genetic and biochemical analysis of *Xanthomonas campestris* chromosomal functions required for xanthan polysaccharide synthesis (xps) was undertaken. Seven xps DNA regions were isolated after conjugation of chemically induced non-mucoid mutants with a genomic library of *X. campestris* DNA. No overlapping segments between regions were detected, based on physical mapping, indicating the unlinked character of these regions. Clones complementing several different mutants belonging to the same region contained overlapping segments of *X. campestris* chromosomal DNA. Complementation and biochemical analysis, and DNA mapping were used to identify and characterize xpsIII, IV and VI DNA regions. Mutants in these three regions were able to synthesize both lipid intermediates and xanthan gum in vitro when sugar nucleotides were provided as substrates. HPLC analysis of the intracellular sugar nucleotide content showed that the XpsIII group comprises two different classes of mutants: XpsIIIA, defective in UDP-glucose, UDP-glucuronic acid and **GDP-mannose**, and XpsIIIB, defective in **GDP-mannose**. XpsIV mutants were defective in UDP-glucose and UDP-glucuronic acid, and XpsVI mutants were defective only in UDP-glucuronic acid. Analysis of enzyme activities involved in the synthesis of UDP-glucose, **GDP-mannose** and UDP-glucuronic acid indicated that the xpsIIIA region affects the activity of the phosphoglucomutase/phosphomannomutase enzyme, and the xpsIIIB region affects the mannoisomerase/phosphomannoisomerase activities. The xpsIV mutations affect the activity of the UDPG-**pyrophosphorylase** enzyme, and the xpsVI mutations affect the activity of the UDPG-dehydrogenase enzyme.

1993

3/3,AB/44 (Item 7 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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08623679 BIOSIS NO.: 199345041754

Identification of amino acids required for the catalytic activities of phosphomannose isomerase-**GDP-mannose pyrophosphorylase** involved in the alginate pathway of *Pseudomonas aeruginosa*.

AUTHOR: May T B; Shinabarger D L; Boyd A; Chakrabarty A M

AUTHOR ADDRESS: Univ. Ill. at Chicago, IL**USA

JOURNAL: Abstracts of the General Meeting of the American Society for Microbiology 93 (0):p272 1993

CONFERENCE/MEETING: 93rd General Meeting of the American Society for
Microbiology Atlanta, Georgia, USA May 16-20, 1993
ISSN: 1060-2011
RECORD TYPE: Citation
LANGUAGE: English
1993

3/3,AB/45 (Item 8 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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08477812 BIOSIS NO.: 199344027812
Purification of **GDP-mannose pyrophosphorylase** and
synthesis of 8-azido-**GDP-mannose**.
AUTHOR: Szumilo T; Drake R R; Szumilo H; Elbein A D
AUTHOR ADDRESS: Dep. Biochemistry, Univ. Arkansas Med. Sciences, Little
Rock, Arkansas 72205**
JOURNAL: Glycobiology 2 (5):p480 1992
CONFERENCE/MEETING: 21st Annual Meeting of the Society for Complex
Carbohydrates, Nashville, Tennessee, USA, November 11-14, 1992.
GLYCOBIOLOGY
ISSN: 0959-6658
RECORD TYPE: Citation
LANGUAGE: English
1992

3/3,AB/46 (Item 9 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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08282638 BIOSIS NO.: 000043048711
SITE-DIRECTED MUTAGENESIS OF PHOSPHOMANNOSE ISOMERASE-**GDP-**
MANNOSE PYROPHOSPHORYLASE INVOLVED IN THE BIOSYNTHESIS OF
ALGINATE BY MUCOID ~~PSEUDOMONAS~~-AERUGINOSA
AUTHOR: MAY T B; SHINABARGER D; BOYD A
AUTHOR ADDRESS: UNIV. ILL., CHICAGO, ILL.
JOURNAL: 92ND GENERAL MEETING OF THE AMERICAN SOCIETY FOR MICROBIOLOGY, NEW
ORLEANS, LOUISIANA, USA, MAY 26-30, 1992. ABSTR GEN MEET AM SOC MICROBIOL
92 (0). 1992. 266. 1992
CODEN: AGMME
DOCUMENT TYPE: Meeting
RECORD TYPE: Citation
LANGUAGE: ENGLISH
1992

3/3,AB/47 (Item 10 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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08048541 BIOSIS NO.: 000093081889
FORMATION OF NUCLEOSIDE DIPHOSPHATE MONOSACCHARIDES NDP-SUGARS BY THE
AGAROPHYTE PTEROCLADIA-CAPILLACEA RHODOPHYCEAE
AUTHOR: MANLEY S L; BURNS D J
AUTHOR ADDRESS: DEP. BIOL., CALIF. STATE UNIV., LONG BEACH, CALIF. 90840.
JOURNAL: J PHYCOL 27 (6). 1991. 702-709. 1991
FULL JOURNAL NAME: Journal of Phycology
CODEN: JPYLA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: The following nucleoside diphosphate monosaccharides (sugar
nucleotides) were identified by HPLC from Pterocladia capillacea Born,

and Thur.: ADP-glucose, UDP-glucose, UDP-D-galactose, and **GDP**-glucose + **mannose**. L-galactose was not identified due to the lack of a standard. Several extraction methods were evaluated for their efficacy. A freeze/thaw (liquid N₂) step followed by formic acid (1 M) extraction, reduced pressure evaporation, and solubilization in water was the preferred method. Differences in media nitrate that resulted in different tissue-N levels (1.8, 2.3, and 3.5% dry wt) and agar yields (34, 31, and 28% dry wt, respectively) also resulted in a marked difference in UDP-D-galactose and ADP-glucose tissue levels (decrease with increasing tissue-N) while the levels of the levels of the other sugar nucleotide agar precursors remained unchanged. Activities of UDP-glucose, **GDP**-glucose, and **GDP**-mannose **pyrophosphorylases**, and UDP-D-glucose-4-epimerase were detected in cell-free extracts using unlabeled and ¹⁴C-labeled substrates. This study strongly supports the proposition that the D-galactose component of agar is synthesized via G-1-P .fwdarw. UDP-glucose .fwdarw. UDP-D-galactose and that the L-galactose component is produced via **mannose**-1-P .fwdarw. **GDP**-mannose .fwdarw. **GDP**-L-galactose.

1991

3/3,AB/48 (Item 11 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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07744797 BIOSIS NO.: 000041053593
LOCALIZATION OF THE PROMOTER REGION CONTROLLING THE PSEUDOMONAS-AERUGINOSA
ALGA GENE
AUTHOR: SHINABARGER D; ZIELINSKI N A; MAY T B; CHAKRABARTY A M
AUTHOR ADDRESS: UNIV. ILL., CHICAGO, ILL.
JOURNAL: 91ST GENERAL MEETING OF THE AMERICAN SOCIETY FOR MICROBIOLOGY
1991, DALLAS, TEXAS, USA, MAY 5-9, 1991. ABSTR GEN MEET AM SOC MICROBIOL 91
(0). 1991. 89. 1991
CODEN: AGMME
DOCUMENT TYPE: Meeting
RECORD TYPE: Citation
LANGUAGE: ENGLISH
1991

3/3,AB/49 (Item 12 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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07525845 BIOSIS NO.: 000091088974
PURIFICATION AND CHARACTERIZATION OF PHOSPHOMANNOSE ISOMERASE-**GDP** D-
MANNOSE PYROPHOSPHORYLASE A BIFUNCTIONAL ENZYME IN THE
ALGINATE BIOSYNTHETIC PATHWAY OF PSEUDOMONAS-AERUGINOSA
AUTHOR: SHINABARGER D; BERRY A; MAY T B; ROTHMEL R; FIALHO A; CHAKRABARTY A
M
AUTHOR ADDRESS: DEP. MICROBIOL. IMMUNOL., UNIVERSITY ILLINOIS COLL. MED.,
835 S. WOLCOTT AVE., CHICAGO, ILL. 60612.
JOURNAL: J BIOL CHEM 266 (4). 1991. 2080-2088. 1991
FULL JOURNAL NAME: Journal of Biological Chemistry
CODEN: JBCHA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: We report here the purification and characterization of
phosphomannose isomerase-guanosine 5'-diphospho-D-**mannose**
pyrophosphorylase, a bifunctional enzyme (PMI-GMP) which catalyzes
both the phosphomannose isomerase (PMI) and guanosine 5'-diphospho-D-
mannose pyrophosphorylase (GMP) reactions of the Pseudomonas
aeruginosa alginate biosynthetic pathway. The PMI and GMP activities

co-eluted in the same protein peak through successive fractionation on hydrophobic interaction exchange, and gel filtration chromatography. The purified enzyme migrated as a 56,000 molecular weight protein on sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and the native protein migrated as a monomer of 54,000 molecular weight upon gel filtration chromatography. The apparent K_m for D-mannose 6-phosphate was 3.03 mM, and the V_{max} was 830 nmol/min/mg of enzyme. For the GMP forward reaction, apparent K_m values of 20.5 μ M and 29.5 μ M for D-mannose 1-phosphate and GTP, respectively, were obtained from double reciprocal plots. The GMP forward reaction V_{max} (5,680 nmol/min/mg of enzyme) was comparable to the reverse reaction V_{max} (5,170 nmol/min/mg of enzyme), and the apparent K_m for GDP-D-mannose was determined to be 14.2 μ M. Both reactions required Mg^{2+} activation, but the PMI reaction rate was 4-fold higher with Co^{2+} as the activator. PMI (but not GMP) activity was sensitive to dithiothreitol, indicating the involvement of disulfide bonds to form a protein structure capable of PMI activity. DNA sequencing of a cloned mutant *algA* gene from *P. aeruginosa* revealed that a point mutation at nucleotide 961 greatly decreased the levels of both PMI and GMP in a crude extract.

1991

3/3,AB/50 (Item 13 from file: 5)
DIALOG(R)File 5:BIOSIS Previews(R)
(c) 2001 BIOSIS. All rts. reserv.

07464501 BIOSIS NO.: 000040044650
BIOSYNTHESIS OF ALGINATE
AUTHOR: NARBAD A; GACESA P; RUSSELL N J
AUTHOR ADDRESS: DEP. BIOCHEM., UNIV. WALES, CARDIFF, UK.
JOURNAL: GACESA, P. AND N. J. RUSSELL (ED.). PSEUDOMONAS INFECTION AND
ALGINATES: BIOCHEMISTRY, GENETICS AND PATHOLOGY. X+233P. CHAPMAN AND HALL:
LONDON, ENGLAND, UK; NEW YORK, NEW YORK, USA. ILLUS. ISBN 0-412-35840-9. 0
(0). 1990. 181-205. 1990
CODEN: 32097
RECORD TYPE: Citation
LANGUAGE: ENGLISH
1990

3/3,AB/51 (Item 14 from file: 5)
DIALOG(R)File 5:BIOSIS Previews(R)
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07330353 BIOSIS NO.: 000090110255
ROLES OF MANGANESE MAGNESIUM AND CALCIUM ON ALGINATE BIOSYNTHESIS BY
~~PSEUDOMONAS~~-AERUGINOSA
AUTHOR: MARTINS L O; BRITO L C; SA-CORREIA I
AUTHOR ADDRESS: LABORATORIO ENGENHARIA BIOQUIMICA, INSTITUTO SUPERIOR
TECNICO, 1096 LISBOA CODEX, PORTUGAL.
JOURNAL: ENZYME MICROB TECHNOL 12 (10). 1990. 794-799. 1990
FULL JOURNAL NAME: Enzyme and Microbial Technology
CODEN: EMTED
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: The presence of optimal concentrations of Mn^{2+} , Mg^{2+} (0.5 mM), and Ca^{2+} (2 mM) in the culture medium avoided the premature stoppage of growth and alginate biosynthesis in *Pseudomonas aeruginosa* and stimulated alginate synthesis. Their role as potential mediators in the control of alginate biosynthesis by a high mucoid *P. aeruginosa* strain appeared not to be attributable to the activation of the synthesis of phosphomannose isomerase (PMI) phosphomannomutase (PMM), GDP-mannose pyrophosphorylase (GMP), or GDP-mannose dehydrogenase

(GMD), the enzyme leading to the synthesis of **GDP-mannuronic acid**, the precursor for polymerization in the alginate pathway. The effect of Mn^{2+} , Mg^{2+} , and Ca^{2+} , as possible activators, was studied in crude extracts of recombinant cells, presenting high enzymatic levels by the overexpression of alginate cloned genes. Mn^{2+} and Mg^{2+} (range 0-20 mM) led to the increase of the enzyme activities assayed in vitro of GMD and PMi, whereas PMM was only activated by Mn^{2+} .

1990

3/3,AB/52 (Item 15 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
(c) 2001 BIOSIS. All rts. reserv.

07125032 BIOSIS NO.: 000039061726

PROPERTIES OF THE BIFUNCTIONAL PHOSPHOMANNOSE ISOMERASE **GDP-MANNOSE PYROPHOSPHORYLASE** ENZYME OF ALGINATE SYNTHESIS IN *PSEUDOMONAS-AERUGINOSA*

AUTHOR: SHINABARGER D; MAY T B; BERRY A

AUTHOR ADDRESS: UNIV. ILL. AT CHICAGO, CHICAGO, ILL.

JOURNAL: 90TH ANNUAL MEETING OF THE AMERICAN SOCIETY FOR MICROBIOLOGY 1990, ANAHEIM, CALIFORNIA, USA, MAY 13-17, 1990. ABSTR ANNU MEET AM SOC MICROBIOL 90 (0). 1990. 227. 1990

CODEN: ASMAC

DOCUMENT TYPE: Meeting

RECORD TYPE: Citation

LANGUAGE: ENGLISH

1990

3/3,AB/53 (Item 16 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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06368708 BIOSIS NO.: 000036071861

EVIDENCE FOR INDEPENDENT DOMAINS IN THE BIFUNCTIONAL ENZYME PHOSPHOMANNOSE ISOMERASE-**GDP MANNOSE PYROPHOSPHORYLASE** IN *PSEUDOMONAS-AERUGINOSA*

AUTHOR: BERRY A; ROTHMEL R K; FIALHO A M; CHAKRABARTY A M

AUTHOR ADDRESS: UNIV. ILL. AT CHICAGO.

JOURNAL: JOINT MEETING OF THE AMERICAN SOCIETY FOR CELL BIOLOGY AND THE AMERICAN SOCIETY FOR BIOCHEMISTRY AND MOLECULAR BIOLOGY, SAN FRANCISCO, CALIFORNIA, USA, JANUARY 29-FEBRUARY 2, 1989. J CELL BIOL 107 (6 PART 3). 1988. 179A. 1988

CODEN: JCLBA

DOCUMENT TYPE: Meeting

RECORD TYPE: Citation

LANGUAGE: ENGLISH

1988

3/3,AB/54 (Item 17 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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05383501 BIOSIS NO.: 000032106630

OVERPRODUCTION OF ALGINATE BIOSYNTHETIC ENZYMES IN *PSEUDOMONAS-AERUGINOSA* BY OVEREXPRESSION OF THE PHOSPHOMANNOSE ISOMERASE PMI GENE

AUTHOR: SA'-CORREIA I; BERRY A; DARZINS A; WANG S-K; CHAKRABARTY A M

AUTHOR ADDRESS: UNIV. ILLINOIS HEALTH SCIENCES CENTER, CHICAGO, ILL. 60612.

JOURNAL: 87TH ANNUAL MEETING OF THE AMERICAN SOCIETY FOR MICROBIOLOGY, ATLANTA, GEORGIA, USA, MARCH 1-6, 1987. ABSTR ANNU MEET AM SOC MICROBIOL 87 (0). 1987. 90. 1987

CODEN: ASMAC

DOCUMENT TYPE: Meeting
RECORD TYPE: Citation
LANGUAGE: ENGLISH
1987

3/3,AB/55 (Item 18 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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04590049 BIOSIS NO.: 000079003086
THE REGULATION OF PROTEOPHOSPHOMANNAN BIOSYNTHESIS IN THE SCP YEAST
CANDIDA-MALTOSA H BY PRECURSOR PREPARATION
AUTHOR: ROEBER B; REUTER G
AUTHOR ADDRESS: FRIEDRICH-SCHILLER-UNIV. JENA, SEKTION BIOL.-MIKROBIELLE
BIOCHEM., DDR-6900 JENA, WOELLNITZER STR. 7.
JOURNAL: Z ALLG MIKROBIOL 24 (5). 1984. 317-328. 1984
CODEN: ZAMMB
RECORD TYPE: Abstract
LANGUAGE: GERMAN

ABSTRACT: C. maltosa H and its mutans H3 and H5 differ in their capacity of mannan biosynthesis, but not in their specific activities of phosphomannose isomerase (PMI), phosphomannose mutase and **GDP-mannose-pyrophosphorylase**. As sole source of C and energy from a mixture of 14C-mannose/glucose or mannose/14C-glucose, C. maltosa H utilizes both hexoses simultaneously especially under O₂-limitation. With mannose as sole source of C and energy the specific activity of PMI increases from 40-90 (aerobic growth) to 550-580 nmol .cntdot. min⁻¹ .cntdot. mg⁻¹ protein (O₂-limitation). Phosphoglucose isomerase shows a similar behavior. PMI purified from glucose-grown cells was inhibited by glyoxylate and fructose-1,6-bisphosphate. The inhibition effect in the biosynthesis of mannose-6-phosphate and its possible role in the regulation of proteophosphomannan biosynthesis are discussed in connection with the affinity of the cell wall for hydrocarbon.

1984

3/3,AB/56 (Item 19 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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04236369 BIOSIS NO.: 000077062414
STUDIES ON SOME ENZYMES OF ALGINIC-ACID BIOSYNTHESIS IN
AZOTOBACTER-VINELANDII GROWN IN CONTINUOUS CULTURE
AUTHOR: HORAN N J; JARMAN T R; DAWES E A
AUTHOR ADDRESS: DEPARTMENT OF CIVIL ENGINEERING, UNIVERSITY OF LEEDS, LEEDS
LS2 9JT, UK.
JOURNAL: J GEN MICROBIOL 129 (10). 1983. 2985-2990. 1983
FULL JOURNAL NAME: Journal of General Microbiology
CODEN: JGMIA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: When a mutant of A. vinelandii was grown in continuous culture, the amount of exocellular polysaccharide produced depended on the dissolved O₂ tension (d.o.t.) and the C source: sucrose supported alginate synthesis in phosphate-limited medium whereas sorbitol did not. Changes in the specific activities of 2 key enzymes of alginate biosynthesis (phosphomannose isomerase and **GDP mannose pyrophosphorylase**), measured in extracts of cells grown with sucrose under a range of d.o.t. values, were reflected by the observed changes in alginate production; the activity of **GDP mannose** dehydrogenase was unchanged. A similar correlation between the specific

activities of these enzymes and the rate of alginate production was observed during a transition from sorbitol to sucrose the sole C source, but in this experiment the activity of **GDP mannose** dehydrogenase also increased with increasing alginate production. After prolonged continuous cultivation on sucrose the mutant gradually lost the ability to produce alginate. The key enzymes of alginate biosynthesis could not be detected in extracts of this nonalginate-producing strain, which had also lost the ability to encyst. Thus, alginate formation is controlled by derepression of key biosynthetic enzymes and alginate evidently plays an important role in encystment.

1983

3/3,AB/57 (Item 20 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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03217563 BIOSIS NO.: 000071030674
SUB CELLULAR LOCALIZATION OF SUGAR NUCLEOTIDE SYNTHETASES
AUTHOR: COATES S W; GURNEY T JR; SOMMERS L W; YEH M; HIRSCHBERG C B
AUTHOR ADDRESS: E. A. DOISY DEP. BIOCHEM., ST. LOUIS UNIV. SCH. MED., ST. LOUIS, MO. 63104, USA.
JOURNAL: J BIOL CHEM 255 (19). 1980. 9225-9229. 1980
FULL JOURNAL NAME: Journal of Biological Chemistry
CODEN: JBCHA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Sugar nucleotides are precursors of sugar moieties in glycolipids and glycoproteins and thus may have a regulatory role in the metabolism of these macromolecules. The subcellular localization of 4 sugar nucleotide synthetases was studied. The **pyrophosphorylases** of **GDP-fucose**, **GDP-mannose** and UDP-glucose were detected only in the cytoplasm; in contrast, at least 85% of the CMP-N-acetylneuraminic acid (CMP-NeuAc) synthetase was found in the nucleus. The latter conclusion was reached by assaying CMP-NeuAc synthetase activity in nuclei and cytoplasm of cells fractionated by a nonaqueous procedure and in karyoplasts and cytoplasts from cells enucleated with Cytochalasin B. In addition, cytoplasts were able to synthesize in situ only 20% as much CMP-NeuAc from free NeuAc as nucleated cells; the same enucleated cells synthesized **GDP-fucose** from free fucose at levels comparable to nucleated cells. The nuclear CMP-NeuAc synthetase activity could not be solubilized by treatment of the nuclei with a mixture of 2% Triton X-100 and 1% sodium deoxycholate, conditions which solubilize both the outer and inner nuclear membranes and the nuclear pores. The activities of CMP-NeuAc synthetase and **GDP-fucose pyrophosphorylase** which are assayed in vitro are probably those catalyzing the reactions in vivo. [Mouse L929 cells, BHK cells and rat liver were used.]

1980

3/3,AB/58 (Item 21 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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02739829 BIOSIS NO.: 000068050429
A CLOSE TEMPORAL AND SPATIAL CORRELATION BETWEEN CELL GROWTH CELL WALL SYNTHESIS AND THE ACTIVITY OF ENZYMES OF MANNAN SYNTHESIS IN ACETABULARIA-MEDITERRANEA
AUTHOR: BACHMANN P; ZETSCHKE K
AUTHOR ADDRESS: INST. ANAT. I, RUHR-UNIV. BOCHUM, POSTFACH 10 21 48, D-4630 BOCHUM 1, W. GER.

JOURNAL: PLANTA (BERL) 145 (4). 1979. 331-338. 1979
FULL JOURNAL NAME: PLANTA (Berlin)
CODEN: PLANA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: The synthesis of cell wall mannan and the activities of guanosine-diphosphate-**mannose-pyrophosphorylase** (EC 2.7.7.13) and mannan synthetase were studied during the development of nucleate and enucleated cells of the alga *A. mediterranea*. The activities of both enzymes are relatively high as long as the cells grow and synthesize mannans. With termination of growth and mannan synthesis, the activities of both enzymes, especially mannan synthetase, drop to a low value. The activities of both enzymes are distributed in the cell along an apical-basal gradient. High activities are present in the apical regions of the cell where growth and mannan synthesis mainly occur. In the basal region, growth, mannan synthesis and the activity of the 2 enzymes are slight. The in vitro activity of **GDP**-Man-pyr is at least 100 times higher than that of mannan synthetase; mannan synthetase activity is apparently the limiting factor in mannan synthesis. This conclusion is supported by the pool sizes of Fru 6-P, Man 6-P, Man 6-P and **GDP**-Man during the development of the cells. The control of mannan synthesis and cell wall formation and growth through the regulation of mannan synthetase activity is discussed.

Set	Items	Description
S1	0	GDP AND MANNOSE AND PYROPHOSPHORYLAS?
S2	89	GDP AND MANNOSE AND PYROPHOSPHORYLAS?
S3	58	RD (unique items)

? s s3 and (antisens? or ribozym?)

	58	S3
	28036	ANTISENS?
	4941	RIBOZYM?
S4	1	S3 AND (ANTISENS? OR RIBOZYM?)

? t s4/3,ab/all

4/3,AB/1 (Item 1 from file: 155)
 DIALOG(R)File 155:MEDLINE(R)
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10053796 99407267

Antisense inhibition of the **GDP-mannose pyrophosphorylase** reduces the ascorbate content in transgenic plants leading to developmental changes during senescence.

Keller R; Renz FS; Kossmann J

Max-Planck-Institut fur Molekulare Pflanzenphysiologie, Golm/Potsdam, Germany.

Plant journal (ENGLAND) Jul 1999, 19 (2) p131-41, ISSN 0960-7412

Journal Code: BRU

Languages: ENGLISH

Document type: JOURNAL ARTICLE

GDP-mannose pyrophosphorylase (GMPase, EC 2.7.7.22) catalyses the synthesis of **GDP-D-mannose** and represents the first committed step in the formation of all guanosin-containing sugar nucleotides found in plants which are precursors for cell wall biosynthesis and, probably more important, the synthesis of ascorbate. A full-length cDNA encoding GMPase from *S. tuberosum* was isolated. Transgenic potato plants were generated in which the GMPase cDNA was introduced in **antisense** orientation to the 35S promoter. Transformants with reduced GMPase activity were selected. Transgenic plants were indistinguishable from the wild-type when held under tissue culture conditions, however, a major change was seen 10 weeks after transfer into soil. Transgenic plants showed dark spots on leaf veins and stems with this phenotype developing from the bottom to the top of the plant. In case of the line with the strongest reduction, all aerial parts finally dried out after 3 months in soil, in contrast to the wild-type plants which did not start to senesce at this time. This coincides with a reduction of ascorbate contents in the transgenic plants, which is in agreement with the recently proposed pathway of ascorbate biosynthesis. Furthermore, leaf cell walls of the transgenic potato plants had **mannose** contents that were reduced to 30-50% of the wild-type levels, whereas the composition of tuber cell walls was unchanged. The glycosylation pattern of proteins was unaffected by GMPase

? b 10, 50, 53, 110, 203, 306, 316, 581

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>>>          316 does not exist
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      08apr01 16:26:46 User242957 Session D246.6
        $3.83    1.196 DialUnits File155
          $7.40   37 Type(s) in Format  4 (UDF)
        $7.40   37 Types
    $11.23 Estimated cost File155
        $5.21    0.930 DialUnits File5
          $13.20   8 Type(s) in Format  3 (UDF)
          $21.45  13 Type(s) in Format  4 (UDF)
        $34.65  21 Types
    $39.86 Estimated cost File5
          OneSearch, 2 files,  2.125 DialUnits FileOS
        $0.35 TYMNET
    $51.44 Estimated cost this search
    $52.00 Estimated total session cost    2.299 DialUnits
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SYSTEM:OS - DIALOG OneSearch
  File 10:AGRICOLA 70-2001/Mar
    (c) format only 2001 The Dialog Corporation
  File 50:CAB Abstracts 1972-2001/Mar
    (c) 2001 CAB International
*File 50: Truncating CC codes is recommended for full retrieval.
See Help News50 for details.
  File 53:FOODLINE(R): Food Science & Technology 1972-2001/Apr 04
    (c) 2001 LFRA
  File 110:WasteInfo 1974-2001/Mar
    (c) 2001 AEA Techn Env.
  File 203:AGRIS 1974-2001/Oct
    Dist by NAL, Intl Copr. All rights reserved
  File 306:Pesticide Fact File 1998/Jun
    (c) 1998 BCPC
*File 306: File has been updated & reloaded. See HELP NEWS 306. New
Bluesheet available in F415 & at URL http://library.dialog.com/bluesheets.
  File 581:Population Demographics 1999/Mar
    (c) 1999 Market Statistics
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Set  Items  Description
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? s gdp and mannose and pyrophosphorylas?

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      1892  GDP
      5581  MANNOSE
      1055  PYROPHOSPHORYLAS?
S1      16  GDP AND MANNOSE AND PYROPHOSPHORYLAS?
? ds
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Set  Items  Description
S1      16  GDP AND MANNOSE AND PYROPHOSPHORYLAS?
? rd
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>>>Duplicate detection is not supported for File 306.
>>>Duplicate detection is not supported for File 581.
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>>>Records from unsupported files will be retained in the RD set.
...completed examining records
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S2 11 RD (unique items)
? s s2 and (antisens? o ribozym?)

11 S2
3231 ANTISENS?
311 RIBOZYM?

S3 1 S2 AND (ANTISENS? OR RIBOZYM?)
? t s2/3,ab/all

>>>No matching display code(s) found in file(s): 306

2/3,AB/1 (Item 1 from file: 10)
DIALOG(R)File 10:AGRICOLA
(c) format only 2001 The Dialog Corporation. All rts. reserv.

3874855 22079298 Holding Library: AGL

Suppression of sorbitol dependence in a strain bearing a mutation in the
SRB1/PSA1/VIG9 gene encoding GDP-mannose pyrophosphorylase%%
% by PDE2 overexpression suggests a role for the Ras/cAMP
signal-transduction pathway in the control of yeast cell-wall biogenesis

Tomlin, G.C. Hamilton, G.E.; Gardner, D.C.J.; Walmsley, R.M.; Stateva,
L.I.; Oliver, S.G.

Reading, U.K. : Society for General Microbiology, c1994-
Microbiology. Sept 2000. v.146 (pt.9) p. 2133-2146.

ISSN: 1350-0872 CODEN: MROBEO

DNAL CALL NO: QR1.J64

Language: English

2/3,AB/2 (Item 2 from file: 10)
DIALOG(R)File 10:AGRICOLA
(c) format only 2001 The Dialog Corporation. All rts. reserv.

3833206 22054336 Holding Library: AGL

Glycosylation deficiency phenotypes resulting from depletion of GDP
-mannose pyrophosphorylase in two yeast species

Warit, S. Zhang, N.; Short, A.; Walmsley, R.M.; Oliver, S.G.; Stateva,
L.I.

UMIST, Manchester, UK.

Oxford : Blackwell Scientific Publications,
Molecular microbiology. June 2000. v. 36 (5) p. 1156-1166.

ISSN: 0950-382X CODEN: MOMIEE

DNAL CALL NO: QR74.M65

Language: English

2/3,AB/3 (Item 3 from file: 10)
DIALOG(R)File 10:AGRICOLA
(c) format only 2001 The Dialog Corporation. All rts. reserv.

3820932 22042242 Holding Library: AGL

Identification of ascorbic acid-deficient Arabidopsis thaliana mutants

Conklin, P.L. Saracco, S.A.; Norris, S.R.; Last, R.L.

Cornell University, Ithaca, NY.

Bethesda, Md. : Genetics Society of America.

Genetics. Feb 2000. v. 154 (2) p. 847-856.

ISSN: 0016-6731 CODEN: GENTAE

DNAL CALL NO: 442.8 G28

Language: English

2/3,AB/4 (Item 4 from file: 10)
DIALOG(R)File 10:AGRICOLA
(c) format only 2001 The Dialog Corporation. All rts. reserv.

3810234 22025758 Holding Library: AGL
Antisense inhibition of the GDP-mannose
pyrophosphorylase reduces the ascorbate content in transgenic plants
leading to developmental changes during senescence
Keller, R. Springer, F.; Renz, A.; Kossmann, J.
Max Planck Institut, Golm, Germany.
Oxford : Blackwell Sciences Ltd.
The Plant journal : for cell and molecular biology. July 1999. v. 19 (2)
p. 131-141.
ISSN: 0960-7412
DNAL CALL NO: QK710.P68
Language: English

GDP-mannose pyrophosphorylase (GMPase, EC 2.7.7.22)
catalyses the synthesis of GDP-D-mannose and represents the
first committed step in the formation of all guanosin-containing sugar
nucleotides found in plants which are precursors for cell wall biosynthesis
and, probably more important, the synthesis of ascorbate. A full-length
cDNA encoding GMPase from *S. tuberosum* was isolated. Transgenic potato
plants were generated in which the GMPase cDNA was introduced in antisense
orientation to the 35S promoter. Transformants with reduced GMPase activity
were selected. Transgenic plants were indistinguishable from the wild-type
when held under tissue culture conditions, however, a major change was seen
10 weeks after transfer into soil. Transgenic plants showed dark spots on
leaf veins and stems with this phenotype developing from the bottom to the
top of the plant. In case of the line with the strongest reduction, all
aerial parts finally dried out after 3 months in soil, in contrast to the
wild-type plants which did not start to senesce at this time. This
coincides with a reduction of ascorbate contents in the transgenic plants,
which is in agreement with the recently proposed pathway of ascorbate
biosynthesis. Furthermore, leaf cell walls of the transgenic potato plants
had mannose contents that were reduced to 30-50% of the wild-type
levels, whereas the composition of tuber cell walls was unchanged. The
glycosylation pattern of proteins was unaffected by GMPase inhibition, as
studied by affinoblot analysis.

2/3,AB/5 (Item 5 from file: 10)
DIALOG(R)File 10:AGRICOLA
(c) format only 2001 The Dialog Corporation. All rts. reserv.

3793124 22010815 Holding Library: AGL
Genetic evidence for the role of GDP-mannose in plant
ascorbic acid (vitamin C) biosynthesis
Conklin, P.L. Norris, S.R.; Wheeler, G.L.; Williams, E.H.; Smirnoff, N.;
Last, R.L.
Boyce Thompson Institute for Plant Research and Section of Genetics and
Development, Cornell University, Ithaca, NY.
Washington, D.C. : National Academy of Sciences,
Proceedings of the National Academy of Sciences of the United States of
America. Mar 30, 1999. v. 96 (7) p. 4198-4203.
ISSN: 0027-8424 CODEN: PNASA6
DNAL CALL NO: 500 N21P
Language: English

Vitamin C (L-ascorbic acid; AsA) acts as a potent antioxidant and
cellular reductant in plants and animals. AsA has long been known to have
many critical physiological roles in plants, yet its biosynthesis is only
currently being defined. A pathway for AsA biosynthesis that features
GDP-mannose and L-galactose has recently been proposed for
plants. We have isolated a collection of AsA-deficient mutants of
Arabidopsis thaliana that are valuable tools for testing of an AsA
biosynthetic pathway. The best-characterized of these mutants (vtc1)
contains approximately equal to 25% of wild-type AsA and is defective in
AsA biosynthesis. By using a combination of biochemical, molecular, and
genetic techniques, we have demonstrated that the VTC1 locus encodes a
GDP-mannose pyrophosphorylase (mannose -1-P

guanyl-transferase). This enzyme provides GDP-mannose, which is used for cell wall carbohydrate biosynthesis and proteoglycosylation as well as for AsA biosynthesis. In addition to genetically defining the first locus involved in AsA biosynthesis, this work highlights the power of using traditional mutagenesis techniques coupled with the Arabidopsis Genome Initiative to rapidly clone physiologically important genes.

2/3,AB/6 (Item 6 from file: 10)
DIALOG(R)File 10:AGRICOLA
(c) format only 2001 The Dialog Corporation. All rts. reserv.

3653669 20627844 Holding Library: AGL

Cloning of the *aceF* gene encoding the phosphomannose isomerase and GDP-mannose pyrophosphorylase activities involved in acetan biosynthesis in *Acetobacter xylinum*

Griffin, A.M. Poelwijk, E.A.; Morris, V.J.; Gasson, M.J.
Amsterdam, The Netherlands : Elsevier Science B.V.

FEMS microbiology letters. Sept 15, 1997. v. 154 (2) p. 389-396.

ISSN: 0378-1097 CODEN: FMLED7

DNAL CALL NO: QR1.F44

Language: English

The *aceF* gene from *Acetobacter xylinum* was identified and cloned from a genomic DNA library. The complete DNA sequence was determined and computer analysis of the translated gene sequence revealed homology with the deduced amino acid sequence of *xanB* from *Xanthomonas campestris*. Therefore *aceF* is likely to encode a bifunctional enzyme with mannose-6-phosphate isomerase (PMI) and GDP-mannose pyrophosphorylase (GMP) activities. PMI and GMP activities were detected in strains of *Escherichia coli* expressing the cloned *aceF* gene.

2/3,AB/7 (Item 7 from file: 10)
DIALOG(R)File 10:AGRICOLA
(c) format only 2001 The Dialog Corporation. All rts. reserv.

3573606 20563600 Holding Library: AGL

Genetic analysis of the transcriptional arrangement of *Azotobacter vinelandii* alginate biosynthetic genes: identification of two independent promoters

Lloret, L. Barreto, R.; Leon, R.; Moreno, S.; Martinez-Salazar, J.; Espin, G.; Soberon-Chavez, G.

Universidad Nacional Autonoma de Mexico, Morelos, Mexico.

Oxford : Blackwell Scientific Publications,

Molecular microbiology. Aug 1996. v. 21 (3) p. 449-457.

ISSN: 0950-382X CODEN: MOMIEE

DNAL CALL NO: QR74.M65

Language: English

The study of alginate biosynthesis, the exopolysaccharide produced by *Azotobacter vinelandii* and *Pseudomonas aeruginosa*, might lead to different biotechnological applications. Here we report the cloning of *A. vinelandii* *algA*, the gene coding for the bifunctional enzyme phosphomannose isomerase-guanosine diphospho-D-mannose pyrophosphorylase (PMI-GMP). This gene was selected by the complementation for xanthan gum production of *Xanthomonas campestris* pv. *campestris* *xanB*-mutants, which lack this enzymatic activity. The complementing cosmid clones selected, besides containing *algA*, presented a gene coding for an alginate lyase activity (*algL*), and some of them also contained *algD* which codes for GDP-mannose dehydrogenase. We present here the characterization of the *A. vinelandii* chromosomal region comprising *algD* and its promoter region, *algA* and *algL*, showing that, as previously reported for *P. aeruginosa*, *A. vinelandii* has a cluster of the biosynthetic alginate genes. We provide evidence for the presence of an *algD*-independent promoter in this region which transcribes at least *algL* and *algA*, and which is regulated in a manner that differs from that of the *algD* promoter.

2/3,AB/8 (Item 8 from file: 10)
DIALOG(R)File 10:AGRICOLA
(c) format only 2001 The Dialog Corporation. All rts. reserv.

3473890 20480978 Holding Library: AGL

Studies on some enzymes of alginic acid biosynthesis in mucoid and nonmucoid *Azotobacter chroococcum* strains

Pecina, A. Paneque, A.

Universidad de Sevilla, Sevilla, Spain.

Totowa, N.J. : Humana Press.

Applied biochemistry and biotechnology. Oct 1994. v. 49 (1) p. 51-58.

ISSN: 0273-2289 CODEN: ABIBDL

DNAL CALL NO: QD415.A1J62

Language: English

Measurements of enzymes involved in alginate biosynthesis were straightforward in mucoid (alginate-positive) *Azotobacter chroococcum* ATCC 4412 crude extracts. At the stationary growth phase, where the production of the exopolysaccharide was greatest, the enzymes phosphomannose isomerase and GDP-mannose pyrophosphorylase increased markedly, whereas phosphomannomutase and GDP-mannose dehydrogenase kept the high activity levels measured in the acceleration growth phase. In nonmucoid (alginate-negative) *A. chroococcum* and *A. vinelandii* strains, the activities of phosphomannose isomerase and GDP-mannose pyrophosphorylase were rather low or, in some cases, undetectables. Except in *A. chroococcum* MCD1, which exhibited a low activity, phosphomannomutase was high in the nonmucoid *Azotobacter* strains, and GDP-mannose dehydrogenase reached a significant activity level in two out of four nonmucoid strains tested. The results suggest that derepression of phosphomannose isomerase and GDP-mannose pyrophosphorylase is a sine qua non condition for alginate formation by *A. chroococcum*.

2/3,AB/9 (Item 9 from file: 10)
DIALOG(R)File 10:AGRICOLA
(c) format only 2001 The Dialog Corporation. All rts. reserv.

3178728 92032857 Holding Library: AGL

Genetics of xanthan production in *Xanthomonas campestris*: the xanA and xanB genes are involved in UDP-glucose and GDP-mannose biosynthesis

Koplin, R. Arnold, W.; Hotte, B.; Simon, R.; Wang, G.; Puhler, A.

Universitat Bielefeld, Bielefeld, Germany

Washington, D.C. : American Society for Microbiology.

Journal of bacteriology. Jan 1992. v. 174 (1) p. 191-199.

ISSN: 0021-9193 CODEN: JOBAAY

DNAL CALL NO: 448.3 J82

Language: English

The nucleotide sequence of a 3.4-kb EcoRI-PstI DNA fragment of *Xanthomonas campestris* pv. *campestris* revealed two open reading frames, which were designated xanA and xanB. The genes xanA and xanB encode proteins of 448 amino acids (molecular weight of 48,919) and 466 amino acids (molecular weight of 50,873), respectively. These genes were identified by analyzing insertion mutants which were known to be involved in xanthan production. Specific tests for the activities of enzymes involved in the biosynthesis of UDP-glucose and GDP-mannose indicated that the xanA gene product was involved in the biosynthesis of both glucose 1-phosphate and mannose 1-phosphate. The deduced amino acid sequence of xanB showed a significant degree of homology (59%) to the phosphomannose isomerase of *Pseudomonas aeruginosa*, a key enzyme in the biosynthesis of alginate. Moreover, biochemical analysis and complementation experiments with the *Escherichia coli* manA fragment revealed that xanB encoded a bifunctional enzyme, phosphomannose isomerase-

GDP-mannose pyrophosphorylase.

2/3,AB/10 (Item 1 from file: 50)
DIALOG(R)File 50:CAB Abstracts
(c) 2001 CAB International. All rts. reserv.

03640871 CAB Accession Number: 981202856

Cloning and sequencing of the Candida albicans homologue of SRB1/PSA1/VIG9, the essential gene encoding GDP-mannose pyrophosphorylase in *Saccharomyces cerevisiae*.

Warit, S.; Walmsley, R. M.; Stateva, E. I.

Department of Biomolecular Sciences, UMIST, PO Box 88, Manchester M60 1QD, UK.

Microbiology (Reading) vol. 144 (9): p.2417-2426

Publication Year: 1998

ISSN: 1350-0872 --

Language: English

Document Type: Journal article

Two genomic fragments were isolated from *C. albicans* which strongly hybridized to SRB1/PSA1/VIG9, an essential gene which encodes GDP-mannose pyrophosphorylase in *S. cerevisiae*. A common 2.5 kb XbaI-PstI fragment was identified, which Southern analysis indicated is most likely unique in the *C. albicans* genome. The fragment contains an ORF, which is 82% identical and 90% homologous to the *Srb1p/Psa1p/Vig9p* from *S. cerevisiae*, contains 1 additional amino acid at position 254 and is able to functionally complement the major phenotypic characteristics of *S. cerevisiae* *srb1* null and conditional mutations. It is concluded that the homologue of SRB1/PSA1/VIG9, named *CaSRB1*, has been cloned and sequenced from *C. albicans*. Northern analysis data indicated that the gene is expressed in *C. albicans* under conditions of growth in the yeast and hyphal form and suggested that its expression might be regulated. 50 ref.

2/3,AB/11 (Item 1 from file: 53)
DIALOG(R)File 53:FOODLINE(R): Food Science & Technology
(c) 2001 LFRA. All rts. reserv.

00814855 FOODLINE ACCESSION NUMBER: 495904

Transgenic plants having early-ripening and completely withering aerial parts.

Kossmann J; Keller R

PATENT ASSIGNEE: Max-Planck-Gesellschaft zur Forderung der Wissenschaften
EV

PATENT: WO 9915674 A2

APPLICATION COUNTRY: DE (DATE(S): 19970919)

PRIORITY APPLICATION DATE: 19960918

DESIGNATED STATES:

See published patent document for Designated Contracting States.

X-REFERENCE: BIOTECHNOLOGY

LANGUAGE: German

SUMMARY LANGUAGE: English

DOCUMENT TYPE: Patent

ABSTRACT: Transgenic plants are disclosed that have stem and leaf portions that ripen rapidly and wither completely without affecting the growth of the underground parts of the plant (i.e. roots and tubers). The genetically modified plants have reduced activity of the enzyme GDP mannose pyrophosphorylase in their cells.
Nucleic acid sequences coding for this enzyme are disclosed. The invention is particularly applicable to potato plants.

? t s3/3,ab/all

>>>No matching display code(s) found in file(s): 306

3/3,AB/1 (Item 1 from file: 10)

3810234 22025758 Holding Library: AGL

Antisense inhibition of the GDP-mannose
pyrophosphorylase reduces the ascorbate content in transgenic plants
leading to developmental changes during senescence

Keller, R. Springer, F.; Renz, A.; Kossmann, J.
Max Planck Institut, Golm, Germany.

Oxford : Blackwell Sciences Ltd.

The Plant journal : for cell and molecular biology. July 1999. v. 19 (2)
p. 131-141.

ISSN: 0960-7412

DNAL CALL NO: QK710.P68

Language: English

GDP-mannose pyrophosphorylase (GMPase, EC 2.7.7.22)
catalyses the synthesis of GDP-D-mannose and represents the
first committed step in the formation of all guanosin-containing sugar
nucleotides found in plants which are precursors for cell wall biosynthesis
and, probably more important, the synthesis of ascorbate. A full-length
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antisense orientation to the 35S promoter. Transformants with reduced
GMPase activity were selected. Transgenic plants were indistinguishable
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major change was seen 10 weeks after transfer into soil. Transgenic plants
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from the bottom to the top of the plant. In case of the line with the
strongest reduction, all aerial parts finally dried out after 3 months in
soil, in contrast to the wild-type plants which did not start to senesce at
this time. This coincides with a reduction of ascorbate contents in the
transgenic plants, which is in agreement with the recently proposed pathway
of ascorbate biosynthesis. Furthermore, leaf cell walls of the transgenic
potato plants had mannose contents that were reduced to 30-50% of the
wild-type levels, whereas the composition of tuber cell walls was
unchanged. The glycosylation pattern of proteins was unaffected by GMPase